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INDIAN AGRICULTURAL  
RESEARCH INSTITUTE, NEW DELHI

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# JOURNAL

OF THE

## ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

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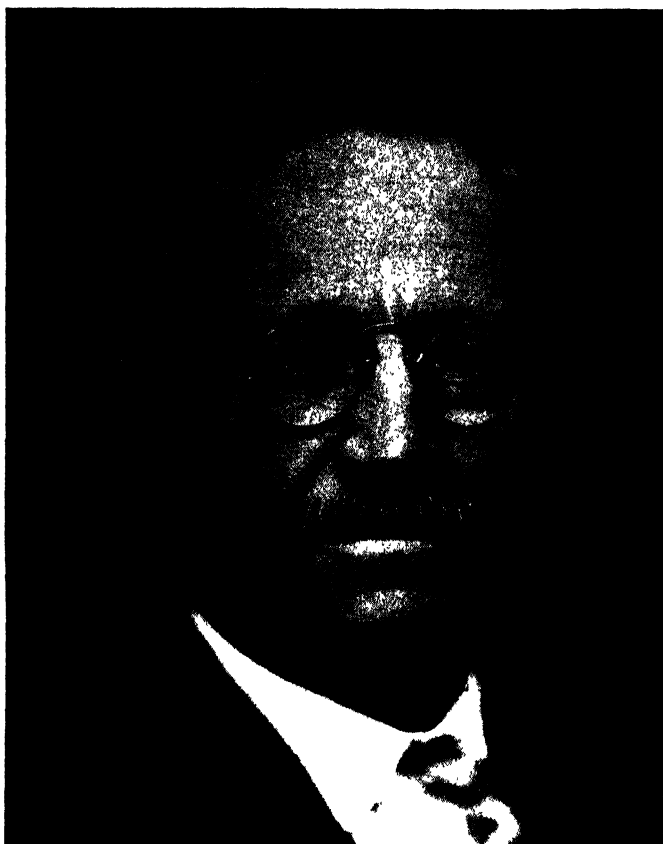
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DR. FLETCHER PEARRE VEITCH, 1868-1943

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## FLETCHER PEARRE VEITCH

The death on October 15, 1943, of Fletcher Pearre Veitch, Collaborator of the Bureau of Agricultural and Industrial Chemistry of the United States Department of Agriculture, marked the passing of one of the country's outstanding agricultural chemists. His 44 years of public service, including 7 with the State of Maryland and 37 with the U. S. Department of Agriculture, gave him a wide and comprehensive knowledge of the chemical problems in the agricultural field.

Dr. Veitch was born on May 22, 1868, in Baltimore, Maryland. He spent his boyhood days on a typical Maryland farm, married a Maryland girl, and lived in Maryland all his life. He experienced the joys as well as the woes of a country lad in helping to market the products of his family's farm, which was 25 miles from Washington, D. C. Those 25-mile rides on a springless hay wagon drawn over a lonely flint-stone road by a 4-horse team trained him to think through his problems in a constructive way, a trait that characterized him as long as he lived.

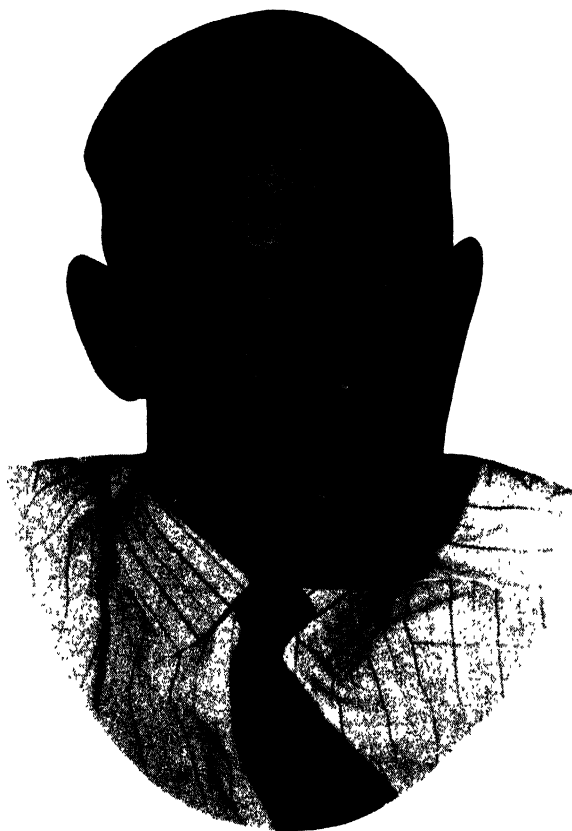
After obtaining all the schooling available in his home community, he attended high school in Washington and later the Maryland Agricultural College (now the University of Maryland), where he was graduated with the B.S. degree in chemistry in 1891. He received the M.S. degree from George Washington University in 1899 and had as his instructor in that institution the late Dr. Harvey W. Wiley, the exponent of the "Pure Food" laws. In 1913, the University of Maryland, in recognition of his many contributions to science, conferred upon him the honorary degree of doctor of science.

Dr. Veitch's professional career began in 1891 when he started work as an assistant to Dr. Milton Whitney, the famous soils expert who was then connected with the Maryland Agricultural Experiment Station. He advanced to various important positions in that institution, ventured into private industry as chemist and superintendent of a fertilizer company for a period of two years, and joined the United States Department of Agriculture in 1901. His advancement in the Department was rapid from soil scientist to Chief of the Leather and Paper Laboratory, Chief of the Industrial Farm Products Research Division, and Chief of the Naval Stores Research Division, where he was serving when he retired from active duty on May 31, 1938. Because of his many years of experience in the industrial utilization of agricultural products, he was retained as a Collaborator in the Bureau where he worked until his death. His research activities covered rosin, turpentine, fertilizers, soils, hides, leathers, tanning materials, textiles, paper and other subjects, and the results of his researches have been reported on under more than 200 titles and have appeared in numerous scientific, technical, and trade publications, and in Department of Agriculture circulars and bulletins. His vision was largely responsible for the establishment of the Department's Naval Stores Station near Lake City, Florida, where research on a pilot-plant scale is conducted on turpentine and rosin.

Dr. Veitch was not only a contributor to science, but he was also an able administrator of scientific work and of the many people who worked under him. He was a member of the American Chemical Society, the American Association for the Advancement of Science (fellow), the International Society of Leather Trades' Chemists, and many other organizations. For many years he had been active in the Association of Official Agricultural Chemists, serving as Vice-president in 1921 and as President in 1922. He also served as Referee on Soils in 1902 and 1903, as Referee on Tanning Materials and Leather from 1907 to 1923 (except the year 1915), and as Referee on Naval Stores from 1924 to his retirement. He was a man with a very distinct personality and believed in hewing to the line of the right regardless of where the chips fell. His hobbies were gardening and hunting. He loved his home and his family, and he will be missed by a host of friends.

C. F. SPEH

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ROBERT HAY KERR, 1881-1943

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## ROBERT HAY KERR

Robert Hay Kerr, for 23 years Senior Chemist in charge of the Meat Inspection Laboratories Section, Meat Inspection Division, formerly of the Bureau of Animal Industry but recently transferred to Livestock and Meats Branch of the Food Distribution Administration, died July 10, 1943, at Hyattsville, Maryland, after an illness of several months.

Mr. Kerr was born in Emporia, Kansas, September 18, 1881, the son of George W. and Mary E. (Ellington) Kerr, and was reared on a farm. He attended Oklahoma Agricultural and Mechanical College, where he received the B.S. degree in chemistry in 1903, and later he had two years of advanced work in chemistry at George Washington University.

From 1903 to 1906 Mr. Kerr was Assistant State Chemist at Maryland Agricultural College (now University of Maryland). On January 22, 1906, he entered the U. S. Department of Agriculture as Scientific Assistant in the Biochemic Division of the Bureau of Animal Industry, but that same year he left the Department of Agriculture to serve as chemist in the Lazaretto Guano Works, Baltimore, Maryland. The next year he returned to the U. S. Department of Agriculture and reentered the Bureau of Animal Industry, where he was engaged for many years in various phases of work in connection with fats and oils. He was placed in charge of the Meat Inspection Laboratories Section of the Meat Inspection Division in 1920, which position he held at the time of his death. He served for a number of years as Referee on Meat and Meat Products for the Association of Official Agricultural Chemists.

Mr. Kerr is survived by his widow; a son, Captain George M. Kerr, of the Veterinary Corps, U. S. Army; and a daughter, Mrs. Marian F. Henderson.

H. R. McMILLIN

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PROCEEDINGS OF THE FIFTY-EIGHTH ANNUAL  
MEETING OF THE ASSOCIATION OF  
OFFICIAL AGRICULTURAL  
CHEMISTS, 1943

The fifty-eighth annual meeting of the Association of Official Agricultural Chemists was held at the Statler Hotel, Washington, D. C., October 27 and 28, 1943.

The meeting was called to order by the president, J. Walter Sale, Food and Drug Administration, Washington, D. C., on the morning of October 27, at 10:00 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES  
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FOR THE YEAR ENDING NOVEMBER, 1944

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PERMANENT COMMITTEES

*Recommendations of Referees*

(Figures in parentheses refer to year in which appointment expires.)

W. F. REINDOLLAR (Bureau of Chemistry, State Department of Health, Baltimore 18, Md.), *Chairman*

SUBCOMMITTEE A: E. L. GRIFFIN (1945) (Office of Distribution, War Food Administration, Washington 25, D. C.), *Chairman*; G. E. GRATTAN (1947), and H. A. HALVORSON (1949).

Feeding stuffs

Sampling

Mineral mixed feeds (calcium and iodine)

Lactose in mixed feeds

Fat in fish meal

Adulteration of condensed milk products

Fat in cooked animal feeds containing cereals

Crude fat or ether extract	Zinc
Filtration aids in crude fiber determination	Copper and cobalt
Soluble chlorine	Chlorophyl, carotene, and iron
Ammoniacal urea and nitrogen salts	Soils and liming materials
Activity of yeast	Hydrogen-ion concentration of soils of arid and semi-arid regions
Microscopic examination	Boron and fluorine
Fluorine	Zinc and copper
Mineral constituents of mixed feeds	Exchangeable calcium and magnesium
Fertilizers	Exchangeable hydrogen
Phosphoric acid (moisture)	Standard solutions
Nitrogen	Sodium thiosulfate solutions
Magnesium and manganese	Thiocyanate solutions
Acid- and base-forming quality	Potassium permanganate solutions
Potash and platinum recovery methods	Buffer solutions
Calcium and sulfur	Vitamins
Copper and zinc	Vitamin A
Insecticides and fungicides	Vitamin B <sub>1</sub>
Fluorine	Vitamin C
D. D. T.	Vitamin D—milk
Rodenticides	Vitamin D—poultry
Nicotine	Vitamin K
Disinfectants	Riboflavin
Leathers and tanning materials	Nicotinic acid
Plants	Carotene and cryptozanthin in yellow corn
Sampling	Panthenic acid
Iodine and boron	
Carbohydrates	

SUBCOMMITTEE B: F. H. WILEY\* (1945) (U.S. Food and Drug Administration, Washington 25, D. C.), *Chairman*; H. J. FISHER (1947), and Dan Dahle (1949).

Naval stores	Methylene blue
Radioactivity	Ethylaminobenzoate
Quantum counter	Metrazol
Vegetable drugs and their derivatives	Barbiturates
Chemical methods for ergot alkaloids	Acetanilid
Theophylline sodium salicylate	Sulfanilamide derivatives
Physostigmine in ointments	Phenolphthalein in presence of bile salts
Quinine ethyl carbonate	Atabrine (chinaclin)
Theobromine and phenobarbital	Sedormid
Prostigmine	Demerol
Aminopyrine, ephedrine, and phenobarbital	Propadrine hydrochloride
Quinine and strychnine	Carbromal
Polarographic methods	Phenolsulfonphthalein
Synthetic drugs	Procaine
Phenothiazine	Sulfobromophthalein
Plasmochine	Miscellaneous drugs
Benzedrine in inhalants	Microchemical tests for alkaloids and synthetics
Hydroxyquinoline sulfate	

\* Unexpired term of W. F. Reindollar.

- Mercury compounds (ethanolamine method)
- Separation of bromides, chlorides, and iodides
- Thyroid
- Emulsions
- Compound ointment of benzoic acid
- Alkali metals
- Spectrophotometric methods
- Glycols and related compounds
- Preservatives and bacteriostatic agents in ampul solutions
- Drug bioassays
  - Enteric coatings
  - Posterior pituitary
  - Ergometrine (ergonovine)
  - Digitalis preparations
- Cosmetics and coal-tar colors
  - Alkalies in cuticle removers
  - Arsenic in hair lotions
  - Lead in cosmetics
  - Mercury salts in cosmetics
  - Pyrogallol in hair dyes
  - Resorcinol in hair lotions
  - Salicylic acid in hair lotions
  - Cosmetic creams
  - Cosmetic powders
- Depilatories
- Deodorants and anti-perspirants
- Urea in deodorants
- Hair dyes and rinses
- Hair straighteners
- Mascara, eyebrow pencils, and eye shadow
- Nail cosmetics
- Moisture in cosmetics
- Ether extract in coal-tar colors
- Pure dye, impurities, and substrata in pigments
- Buffers and solvents in titanium trichloride titrations
- Halogens in halogenated fluoresceins
- Intermediates in certified coal-tar colors
- Spectrophotometric testing of coal-tar colors
- Subsidiary dyes in D&C colors
- Identification of certified coal-tar colors
- Mixtures of coal-tar colors for drug and cosmetic use
- Alizarin in madder lake
- Cosmetic skin lotions
- Acetates, carbonates, halides, and sulfates in certified coal-tar colors

SUBCOMMITTEE C: J. O. CLARKE (1945) (U. S. Food and Drug Administration Chicago 7, Ill.), *Chairman*; C. S. LADD (1947), and KENNETH L. MILSTEAD (1949).

- Canned foods
  - Quality factors
  - Moisture in dried vegetables
- Fill of container methods (foods, drugs, and cosmetics)
- Coffee and tea (chlorogenic acid in coffee)
- Coloring matters in foods
- Dairy products
  - Neutralizers
  - Mold mycelia in butter
  - Lactose in milk
  - Pasteurization of milk and cream
  - Ash in milk and evaporated milk
  - Lactic acid
  - Sampling, fat, and moisture in cheeses
  - Frozen desserts
- Eggs and egg products
  - Unsaponifiable matter and cholesterol
  - Added glycerol and salt
- Fish and other marine products
  - Total solids and ether extract
- Gums in foods
  - Soft curd cheese
  - Mayonnaise and French dressing
  - Frozen desserts
- Meats and meat products
  - Dried skim milk in meat products
  - Soya flour in meat products (immunological tests)
- Metals in foods
  - Selenium
  - Cadmium
  - Copper
  - Zinc
  - Fluorine and lead
  - Preparation of large size samples
  - Mercury



Microbiological methods	Sulfur dioxide in meats
Canned fishery products	Monochloroacetic acid
Canned meats	Spices and condiments
Canned vegetables	Vinegar
Canned tomatoes and other acid vegetable and fruit products	Salad dressings
Sugar	Volatile oil in spices
Eggs and egg products	Moisture and ash in spices
Nuts and nut products	Ash and starch in prepared mustard, starch in mustard flour, and volatile oil in mustard seed
Microchemical methods	Microanalytical methods for extraneous materials
Oils, fats, and waxes	Drugs
Refractometric determination of oil in seeds	Dairy products
Unsataponifiable matter	Nuts and nut products
Peanut oil	Canned foods
Olive oil	Fruits and fruit products
Preservatives and artificial sweeteners	Spices and condiments
Saccharin in non-alcoholic beverages, semi-solid preparations, and baked goods	Vegetable products
Esters of benzoic acid and benzoate of soda	Decomposition in foods
	Volatile acids in fish products
SUBCOMMITTEE D: JOSEPH CALLAWAY (1945*) (U. S. Food and Drug Administration, Washington 25, D. C.), <i>Chairman</i> ; W. CATESBY JONES (1947), and J. WALTER SALE (1949).	
Alcoholic beverages	Chocolate constituents
Malt	Fat
Diastatic activity of malt and alpha- and beta-amylase	Cereal foods (calcium and iron)
Hops	Rye flour in rye bread and in flour mixtures
Cereal adjuncts	H-ion concentration
Brewing sugars and sirups	Starch in raw and cooked cereals
Fermentable extracts in brewing sugars and sirups	Fat acidity-grain, flour, corn meal, and whole wheat flour
Beer	Sugar in bread and other cereal foods
Acidity and pH of beer	Benzoyl peroxide in flour
Inorganic elements in beer	Carbon dioxide in self-rising flour
Color and turbidity in beer	Milk solids and butterfat in bread
Carbon dioxide in beer	Proteolytic activity of flour
Distilled spirits	Soya flour
Spectrophotometric examination of wines and distilled spirits	Soya flour in foods (immunological test)
Formol titrations	Phosphated flour
Chromatographic absorption of wines	Noodles and egg-containing products
pH in distilled alcoholic beverages	Baked products (moisture, ash, protein, fat, and crude fiber)
Wine	Moisture in self-rising flour and in pancake, waffle, and doughnut flours
Cordials and liqueurs	Proteins in flour
Cacao products	
Lecithin in cacao products	
Pectic acid in cacao products	

\* Unexpired term of J. A. LeClerc.

Bromates in flour	Cold pack fruit
Apparent viscosity measurement	Lactic acid and volatile fatty acids
Flavors and non-alcoholic beverages	Sugar and sugar products
B-ionone	Unfermented reducing substances in molasses
Lemon oils and extracts	Drying methods
Organic solvents in flavors	Densimetric and refractometric methods
Glycerol, vanillin, and coumarin in vanilla and imitation vanillas	Honey and honeydew honey
Emulsion flavors	Sucrose and ash in molasses
Maple flavor concentrates and imitations	Confectionery
Diacetyl	Reducing sugars
Fruits and fruit products	Corn sirup and corn sugar
Sodium and chlorides	Color and turbidity in sugar products
Polariscopic methods	Waters, brine, and salt
Titration of acids	Boron in water
Fruit acids	Fluorine in salt
Phosphoric acid	Iodized salt
Potassium	

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A. T. Perkins, Kansas State College, Manhattan, Kans.

LACTOSE IN MIXED FEEDS:

C. W. Sievert, American Dry Milk Institute, Chicago, Ill.

FAT IN FISH MEAL:

Maurice E. Stansby, Fish and Wildlife Service, Seattle 2, Wash.

ADULTERATION OF CONDENSED MILK PRODUCTS:

P. B. Curtis, Agricultural Experiment Station, W. Lafayette, Ind.

FAT IN COOKED ANIMAL FEEDS CONTAINING CEREALS:

S. B. Randle, Agricultural Experiment Station, Lexington, Ky.

CRUDE FAT OR ETHER EXTRACT:

J. J. Taylor, Department of Agriculture, Tallahassee, Fla.

FILTRATION AIDS IN CRUDE FIBER DETERMINATION:

L. S. Walker

SOLUBLE CHLORINE:

J. W. Kuzmeski, Agricultural Experiment Station, Amherst, Mass.

AMMONIACAL UREA AND NITROGEN SALTS:

W. B. Griem, Department of Agriculture, Madison 6, Wisc.

ACTIVITY OF YEAST:

H. J. Witteveen, Department of Agriculture, St. Paul, Minn.

MICROSCOPIC EXAMINATION:

A. W. Creswell, 4806 Winona Terrace, Cincinnati 27, Ohio

FLUORINE:

D. M. Doty, Purdue University, Lafayette, Ind.

MINERAL CONSTITUENTS OF MIXED FEEDS:

J. L. St. John, Agricultural Experiment Station, Pullman, Wash.

FERTILIZERS:

*Referee:* G. S. Fraps, Agricultural Experiment Station, College Station, Tex.

PHOSPHORIC ACID (MOISTURE):

W. H. Ross, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md.

NITROGEN:

A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

MAGNESIUM AND MANGANESE:

J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

ACID- AND BASE-FORMING QUALITY:

H. R. Allen, Agricultural Experiment Station, Lexington, Ky.

POTASH AND PLATINUM RECOVERY METHODS:

O. W. Ford, Agricultural Experiment Station, Lafayette, Ind.

**CALCIUM AND SULFUR:**

Gordon Hart, Department of Agriculture, Tallahassee, Fla.

**COPPER AND ZINC:**

W. Y. Gary, Department of Agriculture, Tallahassee, Fla.

**INSECTICIDES AND FUNGICIDES:**

*Referee:* J. J. T. Graham, Office of Distribution, War Food Administration, Beltsville, Md.

**FLUORINE:**

C. G. Donovan, Office of Distribution, War Food Administration, Beltsville, Md.

**RODENTICIDES:**

John W. Elmore, State Department of Agriculture, Sacramento 14, Calif.

**NICOTINE:**

C. V. Bowen, Bureau of Entomology and Plant Quarantine, Beltsville, Md.

**D.D.T.:**

E. E. Fleck, Bureau of Entomology and Plant Quarantine, Beltsville, Md.

**DISINFECTANTS:**

*Referee:* C. M. Brewer, Office of Distribution, War Food Administration, Beltsville, Md.

**LEATHERS AND TANNING MATERIALS:**

*Referee:* I. D. Clarke, Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Philadelphia 18, Pa.

**PLANTS:**

*Referee:* E. J. Miller, Agricultural Experiment Station, East Lansing, Mich.

**SAMPLING:**

E. J. Miller.

**IODINE AND BORON:**

J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

**CARBOHYDRATES:**

J. T. Sullivan, U. S. Regional Pasture Research Laboratory, State College, Pa.

**ZINC:**

E. J. Benne, Agricultural Experiment Station, East Lansing, Mich.

**COPPER AND COBALT:**

Lillian I. Butler, Agricultural Experiment Station, East Lansing, Mich.

**CHLOROPHYL, CAROTENE, AND IRON:**

E. J. Benne

**SOILS AND LIMING MATERIALS:**

*Referee:* W. H. MacIntire, Agricultural Experiment Station, Knoxville 16, Tenn.

**HYDROGEN-ION CONCENTRATION OF SOILS OF ARID AND SEMI-ARID REGIONS:**

W. T. McGeorge, Agricultural Experiment Station, Tucson, Ariz.

**BORON AND FLUORINE:**

J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

**ZINC AND COPPER:**

Lewis H. Rogers, Agricultural Experiment Station, Gainesville, Fla.

**EXCHANGEABLE CALCIUM AND MAGNESIUM:**

W. M. Shaw, Agricultural Experiment Station, Knoxville 16, Tenn.

**EXCHANGEABLE HYDROGEN:**

W. M. Shaw

**STANDARD SOLUTIONS:**

*Referee:* R. L. Vandaveer, Food and Drug Administration, New Orleans 16, La.

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G. M. Johnson, Food and Drug Administration, St. Louis 1, Mo.

**THIOCYANATE SOLUTIONS:**

E. C. Deal, Food and Drug Administration, New Orleans 16, La.

**POTASSIUM PERMANGANATE SOLUTIONS:**

R. E. Duggan, Food and Drug Administration, New Orleans 16, La.

**BUFFER SOLUTIONS:**

George G. Manov, National Bureau of Standards, Washington 25, D. C.

**VITAMINS:**

*Referee:* Chester D. Tolle, Food and Drug Administration, Washington 25, D. C.

**VITAMIN A:**

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**VITAMIN B<sub>1</sub>:**

O. L. Kline, Food and Drug Administration, Washington 25, D. C.

**VITAMIN C:**

W. L. Hall, Food and Drug Administration, Washington 25, D. C.

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**VITAMIN D—POULTRY:**

Chester D. Tolle

**VITAMIN K:**

H. J. Almquist, University of California, Berkeley, Calif.

**RIBOFLAVIN:**

A. R. Kemmerer, Agricultural Experiment Station, College Station, Tex.

**NICOTINIC ACID:**

To be appointed

**CAROTENE AND CRYPTOZANTHIN IN YELLOW CORN:**

A. R. Kemmerer

**PANTOTHENIC ACID:**

H. W. Loy, Jr., Food and Drug Administration, Washington 25, D. C.

*Subcommittee B*

**NAVAL STORES:**

*Referee:* V. E. Grotlisch, Office of Distribution, War Food Administration, Washington 25, D. C.

**RADIOACTIVITY:**

*Referee:* L. F. Curtiss, National Bureau of Standards, Washington 25, D. C.

**QUANTUM COUNTER:**

A. E. Mix, Food and Drug Administration, Washington 25, D. C.

**ANALYSIS BY RADON MEASUREMENT AND ALPHA PARTICLE COUNTING:**

Francis T. Davis, National Bureau of Standards, Washington 25, D. C.

**VEGETABLE DRUGS AND THEIR DERIVATIVES:**

*Referee:* D. C. Grove, Food and Drug Administration, Washington 25, D. C.

**CHEMICAL METHODS FOR ERGOT ALKALOIDS:**

D. C. Grove

**THEOPHYLLINE SODIUM SALICYLATE:**

To be appointed

**PHYSOSTIGMINE IN OINTMENTS:**

G. M. Johnson, Food and Drug Administration, St. Louis 1, Mo.

**QUININE ETHYL CARBONATE:**

H. G. Underwood, Food and Drug Administration, Chicago 7, Ill.

**THEOBROMINE AND PHENOBARBITAL:**

To be appointed

**PROSTIGMINE:**

F. J. McNall, Food and Drug Administration, Cincinnati 2, Ohio

**AMINOPYRINE, EPHEDRINE, AND PHENOBARBITAL:**

C. D. Wright, Food and Drug Administration, Washington 25, D. C.

**QUININE AND STRYCHNINE:**

To be appointed

**POLAROGRAPHIC METHODS:**

S. Reznick, Food and Drug Administration, Philadelphia 6, Pa.

**SYNTHETIC DRUGS:**

*Referee:* L. E. Warren, Food and Drug Administration, Washington 25, D. C.

**PHENOTHIAZINE:**

V. E. Stewart, Department of Agriculture, Tallahassee, Fla.

**PLASMOCHINE:**

F. C. Sinton, Food and Drug Administration, New York 14, N. Y.

**BENZEDRINE IN INHALANTS:**

J. H. Cannon, Food and Drug Administration, St. Louis 1, Mo.

**HYDROXYQUINOLINE SULFATE:**

A. M. Allison, Food and Drug Administration, Boston 10, Mass.

**METHYLENE BLUE:**

H. O. Moraw, Food and Drug Administration, Chicago 7, Ill.

**ETHYLAMINO BENZOATE:**

To be appointed

**METRAZOL:**

C. B. Stone, Food and Drug Administration, Cincinnati 2, Ohio

**BARBITURATES:**

L. E. Warren.

**ACETANILID:**

To be appointed

**SULFANILAMIDE DERIVATIVES:**

A. E. Sidwell, American Medical Association, Chicago, Ill.

**PHENOLPHTHALEIN IN PRESENCE OF BILE SALTS:**

R. Hyatt, Food and Drug Administration, Cincinnati 2, Ohio

**ATABRINE (CHINACRIN):**

H. C. Heim, Food and Drug Administration, San Francisco, Calif.

**SEDORMID:**

I. Schurman, Food and Drug Administration, Cincinnati 2, Ohio

**DEMEROL:**

To be appointed

**PROPADRINE HYDROCHLORIDE:**

R. D. Stanley, Food and Drug Administration, Chicago 7, Ill.

**CARBROMAL:**

F. A. Rotondaro, Food and Drug Administration, Washington 25, D. C.

**PHENOLSULFONPHTHALEIN:**

L. E. Warren

**PROCAINE:**

Joseph Levine, U. S. Bureau of Narcotics, Washington 25, D. C.

**SULFOBROMOPHTHALEIN:**

F. A. Rotondaro

**MISCELLANEOUS DRUGS:**

*Referee:* C. K. Glycart, Food and Drug Administration, Chicago 7, Ill.

**MICROCHEMICAL TESTS FOR ALKALOIDS AND SYNTHETICS:**

G. L. Keenan, Food and Drug Administration, Washington 25, D. C.

**MERCURY COMPOUNDS (ETHANOLAMINE METHOD):**

P. S. Jorgensen, Food and Drug Administration, San Francisco, Calif.

**SEPARATION OF BROMIDES, CHLORIDES, AND IODIDES:**

N. E. Freeman, Food and Drug Administration, Atlanta 3, Ga.

**THYROID:**

F. A. Rotondaro, Food and Drug Administration, Washington 25, D. C.

**EMULSIONS:**

H. F. O'Keefe, Food and Drug Administration, Chicago 7, Ill.

**COMPOUND OINTMENT OF BENZOIC ACID:**

W. F. Kunke, Food and Drug Administration, Chicago 7, Ill.

**ALKALI METALS:**

W. C. Woodfin, Food and Drug Administration, Atlanta 3, Ga.

**SPECTROPHOTOMETRIC METHODS:**

J. Carol, Food and Drug Administration, Chicago 7, Ill.

**GLYCOLS AND RELATED COMPOUNDS:**

To be appointed

**PRESERVATIVES AND BACTERIOSTATIC AGENTS IN AMPUL SOLUTIONS:**

To be appointed

**DRUG BIOASSAYS:**

*Referee:* R. B. Smith, Food and Drug Administration, Washington 25, D. C.

**ENTERIC COATINGS:**

H. A. Braun, Food and Drug Administration, Washington 25, D. C.

**POSTERIOR PITUITARY:**

R. B. Smith

**ERGOMETRINE (ERGONOVINE):**

B. J. Vos, Jr., Food and Drug Administration, Washington 25, D. C.

**DIGITALIS PREPARATIONS:**

To be appointed

**COSMETICS AND COAL-TAR COLORS:**

*Referee:* Dan Dahle, Food and Drug Administration, Washington 25, D. C.

**ALKALIES IN CUTICLE REMOVERS:**

R. E. Duggan, Food and Drug Administration, New Orleans 16, La.

**ARSENIC IN HAIR LOTIONS:**

H. L. Burrill, Department of Health and Welfare, Augusta, Me.

**LEAD IN COSMETICS:**

W. C. Woodfin, Food and Drug Administration, Atlanta 3, Ga.

**MERCURY SALTS IN COSMETICS:**

To be appointed

**PYROGALLOL IN HAIR DYES:**

C. R. Joiner, Food and Drug Administration, St. Louis 1, Mo.

**RESORCINOL IN HAIR LOTIONS**

F. M. Garfield, Food and Drug Administration, St. Louis 1, Mo.

**SALICYLIC ACID IN HAIR LOTIONS:**

H. R. Bond, Food and Drug Administration, Kansas City 6, Mo.

**COSMETIC CREAMS:**

C. F. Bruening, Food and Drug Administration, Baltimore 2, Md.

**COSMETIC POWDERS:**

George McClellan, Food and Drug Administration, Houston 14, Tex.

**DEPILATORIES:**

F. J. McNall, Food and Drug Administration, Cincinnati 2, Ohio

**DEODORANTS AND ANTI-PERSPIRANTS:**

J. H. Jones, Food and Drug Administration, Baltimore 2, Md.

**UREA IN DEODORANTS:**

N. E. Freeman, Food and Drug Administration, Atlanta 3, Ga.

**HAIR DYES AND RINSES:**

J. H. Jones

**HAIR STRAIGHTENERS:**

J. F. Armstrong, Food and Drug Administration, Los Angeles 15, Calif.

**MASCARA, EYEBROW PENCILS, AND EYE SHADOW:**

Paul W. Jewel, 1666 No. Highland Ave., Hollywood 28, Calif.

**NAIL COSMETICS:**

To be appointed

**MOISTURE IN COSMETICS:**

J. F. Weeks, Food and Drug Administration, New Orleans 16, La.

**COSMETIC SKIN LOTIONS:**

H. R. Bond

**ETHER EXTRACT IN COAL-TAR COLORS:**

S. S. Forrest, Food and Drug Administration, Washington 25, D. C.

**PURE DYE, IMPURITIES, AND SUBSTRATA IN PIGMENTS:**

G. R. Clark, Food and Drug Administration, Washington 25, D. C.

**BUFFERS AND SOLVENTS IN TITANIUM TRICHLORIDE TITRATIONS:**

O. L. Evenson, Food and Drug Administration, Washington 25, D. C.

**HALOGENS IN HALOGENATED FLUORESCENTS:**

G. R. Clark

**INTERMEDIATES IN CERTIFIED COAL-TAR COLORS:**

L. A. Huard, Food and Drug Administration, Washington 25, D. C.

**SPECTROPHOTOMETRIC TESTING OF COAL-TAR COLORS**

S. H. Newburger, Food and Drug Administration, Washington 25, D. C.

**SUBSIDIARY DYES IN D&C COLORS:**

L. Koch, 537 Columbia Street, Brooklyn 31, N. Y.

**IDENTIFICATION OF CERTIFIED COAL-TAR COLORS:**

W. F. Whitmore, Polytechnic Institute, Brooklyn, N. Y.

**MIXTURES OF COAL-TAR COLORS FOR DRUG AND COSMETIC USE:**

W. C. Bainbridge, 537 Columbia Street, Brooklyn 31, N. Y.

**ALIZARIN IN Madder Lake:**

W. C. Bainbridge

**ACETATES, CARBONATES, HALIDES, AND SULFATES IN CERTIFIED COAL-TAR COLORS:**

R. N. Sclar, Food and Drug Administration, Washington 25, D. C.

*Subcommittee C***CANNED FOODS:**

*Referee:* V. B. Bonney, Food and Drug Administration, Washington 25, D. C.

**QUALITY FACTORS:**

To be appointed

**MOISTURE IN DRIED VEGETABLES:**

Henry Fischbach, Food and Drug Administration, Washington 25, D. C.

**FILL OF CONTAINER METHODS (FOODS, DRUGS, AND COSMETICS):**

*Referee:* Sumner C. Rowe, Food and Drug Administration, Washington 25, D. C.



## 12 ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS [Vol. XXVII, No. 1

### COFFEE AND TEA (CHLOROGENIC ACID IN COFFEE):

*Referee:* H. J. Fisher, Agricultural Experiment Station, New Haven 4, Conn.

### COLORING MATTERS IN FOODS:

*Referee:* C. F. Jablonski, Food and Drug Administration, New York 14, N. Y.

### DAIRY PRODUCTS:

*Referee:* G. G. Frary, State Chemical Laboratory, Vermillion, S. D.

#### NEUTRALIZERS:

Fred Hillig, Food and Drug Administration, Washington 25, D. C.

#### MOLD MYCELIA IN BUTTER:

J. D. Wildman, Food and Drug Administration, Washington 25, D. C.

#### LACTOSE IN MILK

E. R. Garrison, University of Missouri, Columbia, Mo.

#### PASTEURIZATION OF MILK AND CREAM:

F. W. Gilcreas, Department of Health, Albany 1, N. Y.

#### ASH IN MILK AND EVAPORATED MILK:

G. G. Frary

#### LACTIC ACID:

Fred Hillig

#### SAMPLING, FAT, AND MOISTURE IN CHEESES:

W. Horwitz, Food and Drug Administration, Minneapolis 1, Minn.

#### FROZEN DESSERTS:

F. Leslie Hart, Food and Drug Administration, Los Angeles 15, Calif.

### EGGS AND EGG PRODUCTS:

*Referee:* To be appointed

#### UNSAAPONIFIABLE MATTER AND CHOLESTEROL:

To be appointed

#### ADDED GLYCEROL AND SALT:

L. C. Mitchell, Food and Drug Administration, Minneapolis 1, Minn.

### FISH AND OTHER MARINE PRODUCTS:

*Referee:* H. D. Grigsby, Food and Drug Administration, Philadelphia 6, Pa.

#### TOTAL SOLIDS AND ETHER EXTRACT:

Manuel Tubis, Food and Drug Administration, Philadelphia 6, Pa.

### GUMS IN FOODS:

*Referee:* F. Leslie Hart, Food and Drug Administration, Los Angeles 15, Calif.

#### SOFT CURD CHEESE:

M. J. Gnagy, Food and Drug Administration, Los Angeles 15, Calif.

#### MAYONNAISE AND FRENCH DRESSING:

S. D. Fine, Food and Drug Administration, Cincinnati 2, Ohio

#### FROZEN DESSERTS:

F. Leslie Hart

### MEAT AND MEAT PRODUCTS:

*Referee:* C. H. Swanger, Bureau of Animal Industry, Washington 25, D. C.

#### DRIED SKIM MILK IN MEAT PRODUCTS:

To be appointed

#### SOYA FLOUR IN MEAT PRODUCTS (IMMUNOLOGICAL TESTS):

C. S. Ferguson, Department of Public Health, Boston, Mass.

### METALS IN FOODS:

*Referee:* H. J. Wichmann, Food and Drug Administration, Washington 25, D. C.

**SELENIUM:**

A. K. Klein, Food and Drug Administration, Washington 25, D. C.

**CADMIUM:**

A. K. Klein

**COPPER:**

C. A. Greenleaf, National Canners' Association, Washington, D. C.

**ZINC:**

L. V. Taylor, American Can Co., Maywood, Ill.

**FLUORINE AND LEAD**

P. A. Clifford, Food and Drug Administration, Washington 25, D. C.

**PREPARATION OF LARGE SIZED SAMPLES:**

P. A. Clifford

**MERCURY:**

J. H. C. Loughrey, Food and Drug Administration, Boston 10, Mass.

**MICROBIOLOGICAL METHODS:**

*Referee:* A. C. Hunter, Food and Drug Administration, Washington 25, D. C.

**CANNED FISHERY PRODUCTS:**

To be appointed

**CANNED MEATS:**

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**CANNED VEGETABLES:**

E. J. Cameron, National Canners' Association, Washington, D. C.

**CANNED TOMATOES AND OTHER ACID VEGETABLE AND FRUIT PRODUCTS:**

B. A. Linden, Food and Drug Administration, Washington 25, D. C.

**SUGAR:**

E. J. Cameron

**EGGS AND EGG PRODUCTS:**

M. T. Bartram, Food and Drug Administration, Washington 25, D. C.

**NUTS AND NUT PRODUCTS:**

To be appointed

**MICROCHEMICAL METHODS:**

*Referee:* To be appointed

**OILS, FATS AND WAXES:**

*Referee:* J. Fitelson, Food and Drug Administration, New York 14, N. Y.

**REFRACTOMETRIC DETERMINATION OF OIL IN SEEDS:**

Lawrence Zeleny, Agricultural Research Center, Beltsville, Md.

**UNSATURATED MATTER:**

Gardner Kirsten, Food and Drug Administration, New York 14, N. Y.

**PEANUT OIL:**

Thomas Riggs, Food and Drug Administration, New York 14, N. Y.

**OLIVE OIL:**

J. Fitelson

**PRESERVATIVES AND ARTIFICIAL SWEETENERS:**

*Referee:* Margarethe Oakley, State Department of Health, Baltimore 18, Md.

**SACCHARIN IN NON-ALCOHOLIC BEVERAGES, SEMI-SOLID PREPARATIONS, AND BAKED GOODS:**

Margarethe Oakley

**ESTERS OF BENZOIC ACID AND BENZOATE OF SODA:**

E. B. Boyce, Department of Public Health, Westfield, Mass.

**SULFUR DIOXIDE IN MEATS:**

C. E. Hynds, State Department of Agriculture and Markets, Albany 1, N. Y.

**MONOCHLORACETIC ACID:**

J. B. Wilson, Food and Drug Administration, Washington 25, D. C.

**SPICES AND CONDIMENTS:**

*Referee:* S. Alfend, Food and Drug Administration, St. Louis 1, Mo.

**VINEGAR:**

A. M. Henry, Food and Drug Administration, Atlanta 3, Ga.

**SALAD DRESSINGS:**

S. D. Fine, Food and Drug Administration, Cincinnati 2, Ohio

**VOLATILE OIL IN SPICES:**

J. F. Clevenger, Food and Drug Administration, New York 14, N. Y.

**MOISTURE AND ASH IN SPICES:**

S. Alfend

**ASH AND STARCH IN PREPARED MUSTARD; STARCH IN MUSTARD FLOUR; AND**

**VOLATILE OIL IN MUSTARD SEED:**

F. M. Garfield, Food and Drug Administration, St. Louis 1, Mo.

**MICROANALYTICAL METHODS FOR EXTRANEEOUS MATERIALS:**

*Referee:* H. Welch, Food and Drug Administration, Washington 25, D. C.

**DRUGS:**

G. L. Keenan, Food and Drug Administration, Washington 25, D. C.

**DAIRY PRODUCTS**

J. D. Wildman, Food and Drug Administration, Washington 25, D. C.

**NUTS AND NUT PRODUCTS:**

F. A. Hodges, Food and Drug Administration, Washington 25, D. C.

**CANNED FOODS:**

K. L. Harris, Food and Drug Administration, Washington 25, D. C.

**FRUITS AND FRUIT PRODUCTS:**

W. G. Helsel, Food and Drug Administration, Washington 25, D. C.

**SPICES AND CONDIMENTS:**

W. V. Eisenberg, Food and Drug Administration, Washington 25, D. C.

**VEGETABLE PRODUCTS:**

F. R. Smith, Food and Drug Administration, Washington 25, D. C.

**DECOMPOSITION IN FOODS:**

*Referee:* W. I. Patterson, Food and Drug Administration, Washington 25, D. C.

**VOLATILE ACIDS IN FISH PRODUCTS:**

Fred Hillig, Food and Drug Administration, Washington 25, D. C.

*Subcommittee D*

**ALCOHOLIC BEVERAGES:**

*Referee:* J. W. Sale, Food and Drug Administration, Washington 25, D. C.

**MALT:**

Christian Rask, Albert Schwill Company, Chicago 17, Ill.

**DIASTATIC ACTIVITY AND ALPHA- AND BETA-AMYLASE OF MALT:**

Allan D. Dickson, University of Wisconsin, Madison 6, Wisc.

**HOPS:**

Frank Rabak, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md.

**CEREAL ADJUNCTS:**

V. E. Munsey, Food and Drug Administration, Washington 25, D. C.

**BREWING SUGARS AND SIRUPS:**

Stephen Laufer, Schwarz Laboratories, Inc., New York 17, N. Y.

**FERMENTABLE EXTRACTS IN BREWING SUGARS AND SIRUPS:**

P. P. Gray, Wallerstein Laboratories, New York, N. Y.

**BEER:**

H. W. Rohde, Schlitz Brewing Company, Milwaukee, Wisc.

**ACIDITY AND pH OF BEER:**

Kurt Becker, Siebel Institute, Chicago, Ill.

**INORGANIC ELEMENTS IN BEER:**

Gordon H. Bendix, Continental Can Company, Chicago 39, Ill.

**COLOR AND TURBIDITY IN BEER:**

B. H. Nissen, Anheuser-Busch, Inc., St. Louis 18, Mo.

**CARBON DIOXIDE IN BEER:**

Irwin Stone, Wallerstein Labs., New York 16, N. Y.

**DISTILLED SPIRITS:**

Peter Valaer, Bureau of Internal Revenue, Washington 25, D. C.

**SPECTROPHOTOMETRIC EXAMINATION OF WINES AND DISTILLED SPIRITS:**

G. F. Beyer, Bureau of Internal Revenue, Washington 25, D. C.

**FORMOL TITRATIONS:**

G. F. Beyer

**CHROMATOGRAPHIC ABSORPTION OF WINES:**

Peter Valaer

**pH IN DISTILLED ALCOHOLIC BEVERAGES:**

M. Rosenblatt, Schenley Research Institute, Inc., Lawrenceburg, Ind.

**WINE:**

G. L. Marsh, University of California, Berkeley 4, Calif.

**CORDIALS AND LIQUEURS:**

J. B. Wilson, Food and Drug Administration, Washington 25, D. C.

**CACAO PRODUCTS:**

*Referee:* W. O. Winkler, Food and Drug Administration, Washington 25, D. C.

**LECITHIN IN CACAO PRODUCTS:**

J. H. Bornmann, Food and Drug Administration, Chicago 7, Ill.

**PECTIC ACID IN CACAO PRODUCTS:**

W. O. Winkler

**CHOCOLATE CONSTITUENTS:**

W. O. Winkler

**FAT:**

Marie L. Offutt, Food and Drug Administration, New York 14, N. Y.

**CEREAL FOODS (CALCIUM AND IRON):**

*Referee:* V. E. Munsey, Food and Drug Administration, Washington 25, D. C.

**RYE FLOUR IN RYE BREAD AND IN FLOUR MIXTURES:**

C. G. Harrel, Pillsbury Flour Mills Company, Minneapolis 15, Minn.

**H-ION CONCENTRATION:**

F. A. Collatz, General Mills, Inc., Minneapolis 15, Minn.

**STARCH IN RAW AND COOKED CEREALS:**

M. P. Etheredge, State Chemical Laboratory, State College, Miss.

**FAT ACIDITY (GRAIN, FLOUR, CORN MEAL, AND WHOLE WHEAT FLOUR):**

Lawrence Zeleny, Agricultural Research Center, Beltsville, Md.

**SUGAR IN BREAD AND OTHER CEREAL FOODS:**

R. M. Sanstedt, Agricultural Experiment Station, Lincoln 1, Nebr.

**BENZOYL PEROXIDE IN FLOUR:**

Dorothy B. Scott, Food and Drug Administration, New York 14, N. Y.

**CARBON DIOXIDE IN SELF-RISING FLOUR:**

R. A. Barackman, Victor Chemical Works, Chicago Heights, Ill.

**MILK SOLIDS AND BUTTERFAT IN BREAD:**

V. E. Munsey

**PROTEOLYTIC ACTIVITY OF FLOUR:**

To be appointed

**SOYA FLOUR:**

W. L. Taylor, General Mills, Inc., Minneapolis 15, Minn.

**SOYA FLOUR IN FOODS (IMMUNOLOGICAL TEST):**

C. S. Ferguson, Department of Public Health, Boston, Mass.

**PHOSPHATED FLOUR:**

Lowell Armstrong, Ballard and Ballard, Louisville, Ky.

**NOODLES AND EGG-CONTAINING PRODUCTS:**

To be appointed

**BAKED PRODUCTS (MOISTURE, ASH, PROTEIN, FAT, AND CRUDE FIBER):**

N. H. Walker, National Biscuit Company, New York, N. Y.

**MOISTURE IN SELF-RISING FLOUR AND IN PANCAKE, WAFFLE, AND DOUGHNUT**

**FLOURS:**

E. F. Steagall, Food and Drug Administration, Washington 25, D. C.

**BROMATES IN FLOUR:**

W. F. Geddes, University of Minnesota, Minneapolis, Minn.

**APPARENT VISCOSITY MEASUREMENT:**

E. G. Bayfield, Kansas State College, Manhattan, Kans.

**FLAVORS AND NON-ALCOHOLIC BEVERAGES:**

*Referee:* J. B. Wilson, Food and Drug Administration, Washington 25, D. C.

**$\beta$ -IONONE:**

J. B. Wilson

**LEMON OILS AND EXTRACTS:**

J. B. Wilson

**ORGANIC SOLVENTS IN FLAVORS:**

R. D. Stanley, Food and Drug Administration, Chicago 7, Ill.

**GLYCEROL, VANILLIN, AND COUMARIN IN VANILLA AND IMITATION VANILLAS:**

Llewellyn Jones, Food and Drug Administration, Kansas City 6, Mo.

**EMULSION FLAVORS:**

J. B. Wilson

**MAPLE FLAVOR CONCENTRATES AND IMITATIONS:**

To be appointed

**DIACETYL:**

J. B. Wilson

**FRUITS AND FRUIT PRODUCTS:**

*Referee:* R. A. Osborn, Food and Drug Administration, Washington 25, D. C.

**SODIUM AND CHLORIDES:**

H. W. Gerritz, Food and Drug Administration, San Francisco, Calif.

**POLARISCOPIC METHODS:**

L. H. McRoberts, Food and Drug Administration, San Francisco, Calif.

**TITRATION OF ACIDS:**

H. M. Bollinger, Food and Drug Administration, Los Angeles 15, Calif.

**FRUIT ACIDS:**

R. A. Osborn

**PHOSPHORIC ACID:**

H. Shuman, Food and Drug Administration, Philadelphia 6, Pa.

**POTASSIUM:**

R. A. Osborn

**COLD PACK FRUIT:**

Paul A. Mills, Food and Drug Administration, Seattle 4, Wash.

**LACTIC ACID AND VOLATILE FATTY ACIDS:**

Fred Hillig, Food and Drug Administration, Washington 25, D. C.

**SUGAR AND SUGAR PRODUCTS:**

*Referee:* C. F. Snyder, National Bureau of Standards, Washington 25, D. C.

**UNFERMENTED REDUCING SUBSTANCES IN MOLASSES:**

F. W. Zerban, N. Y. Sugar Trade Laboratory, 113 Pearl Street, New York 4, N. Y.

**DRYING METHODS:**

Lester D. Hammond, National Bureau of Standards, Washington 25, D. C.

**DENSIMETRIC AND REFRACTOMETRIC METHODS:**

C. F. Snyder

**HONEY AND HONEYDEW HONEY:**

G. P. Walton, Office of Distribution, War Food Administration, Washington 25, D. C.

**SUCROSE AND ASH IN MOLASSES:**

R. A. Osborn, Food and Drug Administration, Washington 25, D. C.

**CONFECTIONERY:**

C. A. Wood, Food and Drug Administration, New York 14, N. Y.

**REDUCING SUGARS:**

Emma J. McDonald, National Bureau of Standards, Washington 25, D. C.

**CORN SIRUP AND CORN SUGAR:**

G. T. Peckham, Jr., Clinton Company, Clinton, Iowa

**COLOR AND TURBIDITY IN SUGAR PRODUCTS:**

J. F. Brewster, National Bureau of Standards, Washington 25, D. C.

**WATERS, BRINE, AND SALT:**

*Referee:* Anna E. Mix, Food and Drug Administration, Washington 25, D. C.

**BORON IN WATER:**

Anna E. Mix

**FLUORINE IN SALT:**

Anna E. Mix

**IODIZED SALT:**

Laura M. Huntley, Michigan Department of Health, Lansing, Mich.

## MEMBERS AND VISITORS PRESENT, 1943 MEETING

Adams, Georgian, Office of Experiment Stations, Washington 25, D. C.  
Allen, Charles D., H. Kohnstamm & Co., Inc., 537 Columbia St., Brooklyn 31, N. Y.  
Allen, H. R., Kentucky Agricultural Experiment Station, Lexington, Ky.  
Allen, Raymond N., Gorton Pew Fisheries Co., Gloucester, Mass.  
Allison, F. E., Bureau of Plant Industry Station, Beltsville, Md.  
Almy, L. H., H. J. Heinz Co., Pittsburgh, Pa.  
Alter, Abraham, Food & Drug Administration, Baltimore 2, Md.  
Anderson, M. S., Bur. Plant Industry, Soils, and Agr. Engineering, Beltsville, Md.

Bacon, C. W., Bureau of Plant Industry, Beltsville, Md.  
Bainbridge, W. C., H. Kohnstamm & Co., Inc., 537 Columbia St., Brooklyn 31, N. Y.  
Baird, Fuller D., Standard Brands, Inc., 595 Madison Ave., New York City  
Balthis, Thos. A., Food Laboratory, 1215 State Office Bldg., Richmond, Va.  
Baringer, John W., Div. Plant Industry, Dept. Agriculture, Columbus, Ohio  
Barnard, H. E., 10th & U Sts., N. W., Washington, D. C.  
Barthen, C. L., White Laboratories, 113 North 13th Street, Newark, N. J.  
Bartlett, Leon E., Park & Pollard Co., Buffalo, N. Y.  
Barton, Raymond W., Mead Johnson & Co., Evansville, Ind.  
Bartram, M. T., Food and Drug Administration, Washington 25, D. C.  
Bastron, Harry, Bur. Animal Industry, Beltsville, Md.  
Batton, H. C., Swift & Co., Fertilizer Works, Baltimore, Md.  
Baumgardner, Robert E., Agricultural Chemist, College Park, Md.  
Beacham, Jr., Lowrie M., Food and Drug Administration, Washington 25, D. C.  
BeMiller, L. N., Mead Johnson & Co., Evansville 21, Ind.  
Berry, Rodney C., 1121 State Office Building, Richmond 1, Va.  
Bevis, C. D., Crisfield, Md.  
Beyer, Geo. F., Bur. Internal Revenue, Washington, D. C.  
Bidez, P. R., State Chemical Laboratory, Auburn, Ala.  
Bittenbender, Conrad D., Fish and Wildlife Service, College Park, Md.  
Blaisdell, Albert C., Bur. Internal Revenue, Washington, D. C.  
Bollinger, George C., 2225 So. Highland Ave., Baltimore 24, Md.  
Bonney, V. B., Food and Drug Administration, Washington 25, D. C.  
Bopst, L. E., University of Maryland, College Park, Md.  
Bowes, F. C., New England By-Products Corp., Boston, Mass.  
Bradford, Z. B., Department of Agriculture, Raleigh, N. C.  
Brewer, Chas. M., Food Distribution Adm., Beltsville, Md.  
Brewster, J. F., National Bureau of Standards, Washington, D. C.  
Broll, Harry R., F. S. Royster Guano Co., Baltimore, Md.  
Broughton, L. B., University of Maryland, College Park, Md.  
Browne, Charles A., Bur. Agr. and Ind. Chemistry, Washington 25, D. C.  
Browne, H. H., Bur. Dairy Industry, Washington 25, D. C.  
Browne, Louise McD., 3408 Lowell St., N. W., Washington, D. C.  
Bruening, Charles F., Food and Drug Administration, Baltimore 2, Md.  
Brunkow, O. R., Commercial Solvents Corp., 17 East 42nd St., New York City  
Bryan, C. S., Rumford Chemical Works, Rumford, R. I.  
Buchanan, P. J., Process Division & Chemical Control, New York City

Carpenter, F. B., Virginia-Carolina Chemical Corp., Richmond, Va.  
Carroll, Jr., E. C., Gorton-Pew Fisheries Co., Gloucester, Mass.  
Carson, Charles T., Frankfort Distilleries, Inc., Box 357, Baltimore, Md.

- Carstenson, Mae E., South Agricultural Building, Washington 25, D. C.  
Carter, R. H., Bur. Entomology & Plant Quarantine, Beltsville, Md.  
Caskey, C. D., 10th Street Building, Richmond 18, Va.  
Chace, Edward M., Bur. Agr. and Ind. Chemistry, Los Angeles 33, Calif.  
Charlton, R. C., American Agricultural Chem. Co., 50 Church St., New York City  
Cherry, Ralph L., Oil, Paint & Drug Reporter, 59 John St., New York City  
Christensen, M. Elmer, 35 State Capitol, Salt Lake City, Utah  
Clark, C. Reynolds, Department of Agriculture, Atlanta, Ga.  
Clark, G. R., Food and Drug Administration, Washington 25, D. C.  
Clarke, J. O., Food and Drug Administration, Chicago 7, Ill.  
Clifford, Paul A., Food and Drug Administration, Washington 25, D. C.  
Collatz, F. A., General Mills, Inc., 200 Chamber of Commerce, Minneapolis, Minn.  
Collins, W. D., U. S. Geological Survey, Washington, D. C.  
Coltrane, D. S., Department of Agriculture, Raleigh, N. C.  
Concannon, C. C., Bur. Foreign and Domestic Commerce, Washington, D. C.  
Constable, E. W., Department of Agriculture, Raleigh, N. C.  
Creswell, A. W., Chemical Products Corporation, Cincinnati, Ohio  
Crittenden, E. D., Solvay Process Co., Hopewell, Va.  
Curtis, P. B., Purdue University, Agricultural Exp. Station, W. Lafayette, Ind.
- Dahle, Dan, Food and Drug Administration, Washington 25, D. C.  
Daniel, Esther P., Food and Drug Administration, Washington 25, D. C.  
Davies, John R., General Foods Corp., 1125 Hudson St., Hoboken, N. J.  
Davis, Russell E., Bur. Animal Industry, Beltsville, Md.  
Davis, R. O. E., Bur. Plant Industry Station, Beltsville, Md.  
Deahle, Neulon, Parke, Davis & Co., Detroit, Mich.  
Deemer, R. B., Bur. Plant Industry, Soils, and Agr. Engineering, Beltsville, Md.  
Delahanty, T. W., Bur. Foreign and Domestic Commerce, Washington, D. C.  
Denton, Charles A., Bur. Animal Industry, Beltsville, Md.  
DeWitt, James B., Food and Drug Administration, Washington 25, D. C.  
Dixon, Harry B., Food Distribution Administration, Beltsville, Md.  
Donovan, C. G., Beltsville Research Center, Beltsville, Md.  
Doty, D. M., Purdue University, Agr. Experiment Station, Lafayette, Ind.  
Drucker, Muriel, Food and Drug Administration, New York City 14.  
Dunbar, P. B., Food and Drug Administration, Washington 25, D. C.  
Dunlap, F. L., Wallace & Tiernan Co., Inc., 809 W. Washington Blvd., Chicago, Ill.
- Ellis, N. R., Bur. Animal Industry, Beltsville, Md.  
Engle, Claude R., 10th & Market Sts., Harrisburg, Pa.  
Etheredge, M. P., Box 283, State College, Miss.  
Evenson, O. L., Food and Drug Administration, Washington 25, D. C.
- Fields, S. A., Board of Agriculture, McPherson, Kans.  
Fischbach, Henry, Food and Drug Administration, Washington 25, D. C.  
Fisher, Harry J., Agr. Experiment Station, New Haven 4, Conn.  
Fleck, Elmer E., Bur. Entomology & Plant Quarantine, Beltsville, Md.  
Fleming, Christian, Clinton St. & Mertens Ave., Baltimore, Md.  
Forrest, S. S., Food and Drug Administration, Washington 25, D. C.  
Fox, Mary C., Davison Chemical Corp., Baltimore, Md.  
Frery, Guy G., State Chemical Laboratory, Vermillion, S. D.  
Fritz, James C., Borden Co., Elgin, Ill.  
Fuqua, V. L., 502 State Office Bldg., Nashville, Tenn.



Gaddy, V. Lesessne, Bur. Plant Industry, Soils & Agr. Eng., Beltsville, Md.  
 Geagley, W. C., Department of Agriculture, Lansing, Mich.  
 Gensler, Howard E., 338 Chestnut St., Harrisburg, Pa.  
 Goodwin, M. W., 2101 E. Fort Ave., Baltimore, Md.  
 Gottschall, Gertrude, Food Distribution Administration, Washington 25, D. C.  
 Goulden, Harold D., Toilet Goods Association, 9 Rockefeller Plaza, New York City  
 Gourley, C. O., Beacon Milling Co., Inc., Cayuga, N. Y.  
 Graham, J. J. T., Food Distribution Administration, Beltsville, Md.  
 Green, F. W., Nat. Aniline Div., Allied Chemical & Dye Corp., New York City  
 Greene, E. Peck, Agricultural Building, Tallahassee, Fla.  
 Griem, W. B., Department of Agriculture, Madison 6, Wis.  
 Griffin, E. L., Food Distribution Administration, Washington 25, D. C.  
 Grove, Donald C., Food and Drug Administration, Washington 25, D. C.  
 Guthrie, John D., Southern Regional Research Laboratory, New Orleans 19, La.

Hall, Wallace L., Food and Drug Administration, Washington 25, D. C.  
 Hammond, Lester D., National Bureau of Standards, Washington, D. C.  
 Hand, W. F., State College, Miss.  
 Hanson, H. H., Dover, Del.  
 Hardesty, John O., Bur. Plant Industry, Soils & Agr. Engineering, Beltsville, Md.  
 Harris, Benjamin R., Epstein, Reynolds & Harris, 5 So. Wabash Ave., Chicago, Ill.  
 Hart, Gordon, Department of Agriculture, Tallahassee, Fla.  
 Haskins, Arthur L., Pennsylvania State College, State College, Pa.  
 Hennessy, Edward J., Office of Price Administration, Washington 25, D. C.  
 Hillhouse, F. B., Bureau of Foreign and Domestic Commerce, Washington 25, D. C.  
 Hillig, Fred, Food and Drug Administration, Washington 25, D. C.  
 Hinkle, S. F., Hershey Chocolate Corp., Hershey, Pa.  
 Holland, J. Rich, Wiley & Co., 904 No. Calvert St., Baltimore, Md.  
 Holley, K. T., Agricultural Experiment Station, Experiment, Ga.  
 Holmes, R. S., Bur. Plant Industry, Soils, and Agr. Engineering, Beltsville, Md.  
 Hoover, G. W., 2121 Virginia Ave., N. W., Washington, D. C.  
 Hord, E. T., Department of Agriculture, Raleigh, N. C.  
 Howes, C. Clifton, Davison Chemical Corp., Baltimore, Md.  
 Huard, Leo A., Food and Drug Administration, Washington 25, D. C.  
 Huber, Wolfgang, Winthrop Chemical Co., Rensselaer, N. Y.  
 Hughes, Harris, Hughes & Co., Irvington, Ky.  
 Hunter, Albert C., Food and Drug Administration, Washington 25, D. C.  
 Huntley, Laura M., Department of Health, Lansing 4, Mich.  
 Huston, H. A., 83-09 Talbot Place, Kew Gardens, N. Y.

Ijams, Paul, Board of Agriculture, Topeka, Kans.  
 Ingle, Mark J., Richardson Corp., 1069 Lyell Ave., Rochester, N. Y.  
 Irish, Fred W., Federal Trade Commission, Washington 25, D. C.

Jacob, K. D., Bur. Plant Industry, Soils, and Agr. Engineering, Beltsville, Md.  
 Jacobs, Carl B., Davison Chemical Corp., Baltimore, Md.  
 Jarvis, N. D., Fish and Wildlife Service, College Park, Md.  
 Jatul, Bernard, Sharp and Dohme, Glenolden, Pa.  
 Johnson, Emmet B., Ralston Purina Co., St. Louis, Mo.  
 Jones, Howard T., Distilled Spirits Inst., National Press Bldg., Washington, D. C.  
 Jones, John H., Food and Drug Administration, Washington 25, D. C.  
 Jones, W. Catesby, Department of Agriculture, Richmond 1, Va.  
 Jones, W. Parker, Union Trust Bldg., Washington, D. C.

Jukes, T. H., Lederle Laboratories, Pearl River, N. Y.  
Jurist, Benjamin, Food and Drug Section, Office Price Adm., Washington 25, D. C.

Kauffman, W. R., Bur. Animal Industry, Beltsville, Md.  
Kebler, Lyman F., 1322 Park Road, Washington, D. C.  
Keenan, George L., Food and Drug Administration, Washington 25, D. C.  
Keenen, Frank G., DuPont Company, Wilmington, Del.  
Kellogg, James W., Jodine Educational Bureau, 75 E. Wacker Drive, Chicago, Ill.  
Kellogg, W. L., Bur. Animal Industry, Beltsville, Md.  
Kennedy, George H., E. I. DuPont & Company, New Brunswick, N. J.  
Kenney, Frank, Walter Baker & Co., Inc., Boston, Mass.  
Kerr, A. P., Agricultural Experiment Station, Baton Rouge, La.  
Kiester, J. T., 827 F St., N. E., Washington, D. C.  
Kinney, C. N., Drake University, Des Moines, Iowa  
Klein, Alfred K., Food and Drug Administration, Washington 25, D. C.  
Kline, O. L., Food and Drug Administration, Washington 25, D. C.  
Kneeland, Jr., Ralph F., Food and Drug Administration, Washington 25, D. C.  
Knight, Howard Lawton, Experiment Station Record, Washington 25, D. C.  
Knudsen, Lila F., Food and Drug Administration, Washington 25, D. C.  
Koch, Louis, H. Kohnstamm & Co., 537 Columbia Street, Brooklyn 31, N. Y.  
Koff, Arnold C. D., Smith Pharmacal Co., Orange, N. J.  
Kokoski, Frank J., Agricultural Experiment Station, Geneva, N. Y.  
Kraybill, H. R., American Meat Institute, 59 E. Van Buren Street, Chicago, Ill.  
Kreiling, R. G., Armour Fertilizer Works, Atlanta, Ga.

Ladd, Culver S., War Food Adm., Food Distribution Adm., Washington 25, D. C.  
Lancaster, H. M., Department of Pensions and National Health, Ottawa, Canada  
Lapp, Marian E., Association of Official Agricultural Chemists, Washington 4, D. C.  
Laufer, Stephen, Schwarz Laboratories, 202 East 44th Street, New York City  
Leatherman, P. K., Emerson Drug Company, Baltimore, Md.  
Leavell, Gladys, Bur. Animal Industry, Beltsville, Md.  
LeClerc, J. A., 225 Cedar Avenue, Takoma Park, Md.  
Lee, Clarence E., Beacon Milling Co., Inc., Cayuga, N. Y.  
Leighty, W. R., Bur. Plant Industry, Soils, and Agr. Engineering, Beltsville, Md.  
Lepper, Henry A., Food and Drug Administration, Washington 25, D. C.  
Lindahl, Ivan, National Research Center, Beltsville, Md.  
Linder, William V. Alcohol Tax Unit, Washington, D. C.  
Lodge, F. S., National Fertilizer Assoc., Investment Building, Washington, D. C.  
Long, Malcolm A., Atlantic Supply Co., 1000 Hull Street, Baltimore, Md.  
Loy, Jr., Henry W., Food and Drug Administration, Washington 26, D. C.  
Luckmann, Frederick H., Best Foods, Inc., 99 Avenue A, Bayonne, N. J.  
Ludwick, R. W., New Mexico State College, State College, N. M.  
Lythgoe, Hermann C., Department of Public Health, State House, Boston, Mass.  
Lythgoe, Mrs. H. C. 36 Fair Oaks Avenue, Newtonville, Mass.

MacIntire, W. H., Agricultural Experiment Station, Knoxville, Tenn.  
Macomber, Hugh I., Food and Drug Administration, Baltimore 2, Md.  
Magruder, E. W., F. S. Royster Guano Co., Norfolk, Va.  
Mann, R. F., White Laboratories, 113 North 13th Street, Newark, N. J.  
Markwood, L. N., Bur. Foreign and Domestic Commerce, Washington 25, D. C.  
Marshall, Ogden E., Fleischmann Laboratories, New York City, 51  
Martinek, William A., Fisheries Technological Laboratory, College Park, Md.  
Matlack, M. B., Food Distribution Adm., Washington 25, D. C.

- Matthews, Wilbur P., Department of Agriculture, Raleigh, N. C.  
 Maynard, Wayne E., Fleischmann Laboratories, New York City, 51  
 Mehurin, R. M., Food Distribution Adm., Washington 25, D. C.  
 Merrill, Edward C., United Drug Company, 93 Leon Street, Boston, Mass.  
 Miller, Charles O., Bur. Animal Industry, Beltsville, Md.  
 Miller, Glennard E., Fleischmann Laboratories, New York City, 51  
 Miller, R. C. Pennsylvania State College, State College, Pa.  
 Mitchell, H. S., Swift and Company, Chicago, Ill.  
 Mix, Anne E., Food and Drug Administration, Washington 25, D. C.  
 Molitor, J. Clinton, Food and Drug Administration, Washington 25, D. C.  
 Morgareidge, Kenneth, National Oil Products Co., Harrison, N. J.  
 Morris, Harold P., U. S. Public Health Service, Bethesda, Md.  
 Moyer, T. R., Cooperative Fertilizer Research Bur., 2101 E. Fort Ave., Baltimore, Md.  
 Munsey, Virdell E., Food and Drug Administration, Washington 25, D. C.  
 McAdams, Harry C., E. F. Drew Company, 919 No. Michigan Ave., Chicago, Ill.  
 McCall, A. G., State Programs Officer, Washington 25, D. C.  
 McCallister, Jr., J. G., Baugh Chemical Company, Baltimore, Md.  
 McDonald, Emma J., National Bureau of Standards, Washington 25, D. C.  
 McDonnell, C. C., Insecticide Div., Food Distribution Adm., Washington 25, D. C.  
 McDonnell, H. B., College Park, Md.  
 McNally, Edmund, Bur. Animal Industry, Beltsville, Md.
- Nantz, John D., National Aniline Div., Allied Chemical and Dye Corp., Buffalo, N. Y.  
 Neff, Carroll F., Ironized Yeast Laboratories, 611 Forrest Road, N. E., Atlanta, Ga.  
 Nelson, E. M., Food and Drug Administration, Washington 25, D. C.  
 Nelson, Franklin C., Stanco, Inc., 216 West 14th St., New York City  
 Nelson, J. Wesley, Cargill, Inc., Minneapolis, Minn.  
 Neutzel, Carl, P. O. Box 1643, Baltimore, Md.  
 Newburger, Sylvan H., Food and Drug Administration, Washington 25, D. C.
- Oakley, Margarethe, State Department of Health, Baltimore 18, Md.  
 Obbink, Henry, Bur. Foods and Dairy, State House, Lincoln, Nebr.  
 Offutt, Marie L., Food and Drug Administration, New York City, 14  
 Osborn, R. A., Food and Drug Administration, Washington 25, D. C.
- Palmore, Julian I., Food and Drug Administration, Washington 25, D. C.  
 Parker, Hugh K., Wallace and Tiernan, Newark, N. J.  
 Parsons, J. T., H. J. Heinz Company, Pittsburgh, Pa.  
 Patterson, E. B., Arthur H. Thomas Company, Philadelphia 5, Pa.  
 Patterson, H. J., College Park, Md.  
 Patterson, Wilbur I., Food Div., Food Distribution Adm., Washington 25, D. C.  
 Peckham, Jr., George T., Clinton Company, Clinton, Iowa  
 Pederson, S. A., A. & P. Tea Company, 711 W. Lake Street, Minneapolis, Minn.  
 Phillips, Thomas G., University of New Hampshire, Durham, N. H.  
 Pickering, H. F., N. C. Department of Agriculture, Raleigh, N. C.  
 Porter, William L., Eastern Regional Research Lab., Philadelphia 18, Pa.  
 Pottinger, S. R., Fish and Wildlife Service, College Park, Md.  
 Prebluda, H. J., U. S. Industrial Chemicals, Inc., 60 E. 42nd St., New York City  
 Prevol, E. R., Fertilizer Division, Caldwell, Idaho  
 Price, T. M., Municipal Building, Washington, D. C.

Quackenbush, F. W., Purdue University, Lafayette, Ind.

Queen, W. A., Food and Drug Administration, Washington 25, D. C.

Quillen, J. W., Bur. Internal Revenue, Apprisers Stores, Baltimore, Md.

Rabak, Frank, Bur. Plant Industry, Soils, and Agr. Engineering, Beltsville, Md.

Rader, Lewis F., Bur. Plant Industry, Soils, and Agr. Engineering, Beltsville, Md.

Ramsay, Lessel L., Food and Drug Administration, Washington 25, D. C.

Randle, Stacy B., Agricultural Experiment Station, Lexington, Ky.

Reed, John B., D. C. Health Dept., New Municipal Building, Washington, D. C.

Reichen, Laura, Food and Drug Administration, Washington 25, D. C.

Reindollar, W. F., State Health Department, Baltimore 18, Md.

Reznek, S., Food and Drug Administration, Philadelphia 6, Pa.

Rhodes, L. B., Department of Agriculture, Raleigh, N. C.

Roark, R. C., Bur. Entomology and Plant Quarantine, Beltsville, Md.

Robb, J. Bernard, A.B.C. Board, Richmond, Va.

Robertson, A. H., Department of Agriculture and Markets, Albany, N. Y.

Robinson, W. O., Bur. Plant Industry, Soils, and Agr. Engineering, Beltsville, Md.

Roe, Joseph H., George Washington University, Washington, D. C.

Romaine, J. D., American Potash Institute, Washington 6, D. C.

Ross, William H., Bur. Plant Industry, Soils, and Agr. Engineering, Beltsville, Md.

Rontondaro, F. A., Food and Drug Administration, Washington 25, D. C.

Rowe, Sumner C., Food and Drug Administration, Washington 25, D. C.

Rust, Walter A., Fish and Wildlife Service, College Park, Md.

Rynasiewicz, Joseph, R. I. State College Experiment Station, Kingston, R. I.

Sale, J. Walter, Food and Drug Administration, Washington 25, D. C.

Sanford T. D., F. E. Booth Company, Inc., 129 Powell Street, Emeryville, Calif.

Sankowsky, N. A., Stanco, Inc., P. O. Box 216, Elizabeth, N. J.

Sauchelli, Vincent, Davison Chemical Corp., Baltimore, Md.

Schneider, Roy, National Institute of Health, Bethesda, Md.

Scholes, John C., Beacon Milling Co., Inc., Cayuga, N. Y.

Schreiner, Oswald, Bur. Plant Industry, Soils, and Agr. Engineering, Beltsville, Md.

Seif, L. Dale, Wm. S. Merrill Co., Cincinnati, Ohio

Sherman, Mrs. Mildred S., Bur. Plant Industry, Soils, and Agr. Engineering, Beltsville, Md.

Sherwood, R. C., War Food Administration, Washington 25, D. C.

Shuey, Philip McG., Shuey & Company, 11 East Bay Street, Savannah, Ga.

Shupe, Irwin S., Winthrop Chemical Co., 33 Riverside Avenue, Rensselaer, N. Y.

Sidwell, Jr., Albert E., American Medical Association, Chicago, Ill.

Siebel, Jr., F. P., J. E. Siebel Sons Company, Chicago, Ill.

Siems, H. B., Swift and Company, Fertilizer Department, Chicago 9, Ill.

Sipherd, I. R., National Distillers, 120 Broadway, New York City

Skinner, W. W., Bur. Agricultural and Industrial Chemistry, Washington 25, D. C.

Smith, Arthur M., Synthetic Nitrogen Products Corp., 285 Madison Ave., New York City

Smith, C. A., Standard Brands, 595 Madison Ave., New York 22, N. Y.

Smith, Herbert J., Ralston Purina Co., St. Louis, Mo.

Smith, John B., R. I. State College, Kingston, R. I.

Smith, M. I., National Institute of Health, Bethesda, Md.

Snider, James B., Civil Service Commission, Washington, D. C.

Snyder, Carl F., National Bureau of Standards, Washington, D. C.

Snyder, E. F., Civil Service Commission, Washington, D. C.  
 Snyder, Roy K., National Institute of Health, Bethesda, Md.  
 Speh, Carl F., Bur. Agricultural and Industrial Chemistry, Washington 25, D. C.  
 Spurr, Frank A., Beltsville, Md.  
 Steagall, Edward F., Food and Drug Administration, Washington 25, D. C.  
 Stevens, Henry, Bur. Agricultural & Engineering Chemistry, Washington 25, D. C.  
 Stewart, R. R., Washington, D. C.  
 Struve, Oscar, Eastern States Coop. Milling Corp., Buffalo 5, N. Y.  
 Suggs, George W., Barrett Division, Allied Chemical and Dye Corp., Atlanta, Ga.  
 Sullivan, Betty, Russell Miller Milling Co., Minneapolis, Minn.  
 Swanger, R. K., Food Distribution Administration, Washington 25, D. C.

Taylor, J. N., Bur. Foreign and Domestic Commerce, Washington, D. C.  
 Taylor, L. V., American Can Company, Maywood, Ill.  
 TenEyck, H. S., Southern Phosphate Corp., Bartow, Fla.  
 Thompson, Ernest C., Borden Company, 350 Madison Ave., New York City  
 Titus, Harry W., Bur. Animal Industry, Beltsville, Md.  
 Tobey, Elmer R., Agricultural Experiment Station, Orono, Me.  
 Tolle, Chester D., Food and Drug Administration, Washington 25, D. C.  
 Tolman, L. M., Wilson and Company, Chicago, Ill.  
 Tremearne, Thomas H., War Production Board, Washington 25, D. C.  
 Tressler, Donald K., General Electric Company, Bridgeport, Conn.  
 Trimble, Charles E., 2272 South Clinton Street, Baltimore, Md.  
 Turner, J. D., Agricultural Experiment Station, Lexington, Ky.  
 Turrentine, J. W., American Potash Institute, Washington 6, D. C.

Vahlteich, H. W., Best Foods, Inc., Bayonne, N. J.  
 Valaer, Jr., Peter, Bur. Internal Revenue, Washington 25, D. C.  
 Vliet, Elmer B., Abbott Laboratories, North Chicago, Ill.  
 Vollertsen, J. J., Armour & Company, U. S. Yards, Chicago, Ill.

Waddell, James, E. I. DuPont de Nemours & Company, New Brunswick, N. J.  
 Wales, H., Food and Drug Administration, Washington 25, D. C.  
 Walker, L. S., Agricultural Experiment Station, Burlington, Vt.  
 Walton, G. P., Food Distribution Adm., Washington 25, D. C.  
 Warren, L. E., Food and Drug Administration, Washington 25, D. C.  
 Waterman, Henry C., Office of Experiment Stations, Washington 25, D. C.  
 Webb, H. J., Clemson College, Clemson, S. C.  
 Weiss, Francis Joseph, National Planning Assoc., Washington, D. C.  
 Wells, Eugene H., Food and Drug Administration, Washington 25, D. C.  
 Welsh, Llewellyn, H., Food and Drug Administration, Washington 25, D. C.  
 Westfall, Benton B., National Cancer Institute, Bethesda, Md.  
 White, W. B., Food and Drug Administration, Washington 25, D. C.  
 Whitney, W. T., Coronet Phosphate Company, Plant City, Fla.  
 Wichmann, H. J., Food and Drug Administration, Washington 25, D. C.  
 Wigley, A. B., Phipps and Bird, Inc., Richmond, Va.  
 Wilkie, J. B., Food and Drug Administration, Washington 25, D. C.  
 Williams, Victor B., Missouri Department of Agriculture, Jefferson City, Mo.  
 Willis, R. L., Agricultural Experiment Station, New Brunswick, N. J.  
 Willits, Charles O., Eastern Regional Research Laboratory, Philadelphia 18, Pa.  
 Wilson, John B., Food and Drug Administration, Washington 25, D. C.  
 Wilson, Stansbury M., Baugh Chemical Co., Baltimore, Md.

Winkler, William O., Food and Drug Administration, Washington 25, D. C.

Winton, Andrew L., Wilton, Conn.

Witt, J. C., Marquette Cement Mfg. Co., Chicago, Ill.

Wood, A. P., Solvay Process Co., Syracuse, N. Y.

Zeigler, C. C., Swift and Company, Chicago, Ill.

Zinzalian, George, 416 Division Street, Boonton, N. J

# PRESIDENT'S ADDRESS<sup>1</sup>

## SOME HIGH LIGHTS OF WAR-TIME FOOD

By J. WALTER SALE (U. S. Food and Drug Administration,  
Washington, D. C.)

Members and Visitors of the A. O. A. C.:

We are assembled here for the second war meeting of the Association in a period of 58 years, the first of such meetings having been held in 1917 during the first year of World War I. Last year, as in 1918, we deferred our usual annual meeting. It was not considered to be practicable, however, to postpone the meeting for the second time in succession, owing to the necessity of transacting accumulated business essential to the welfare of the Association and of the groups that it serves.

While the history and functions of our Association have been extensively discussed in the presidential addresses of my predecessors, I should like, in the interest of our newer members, to devote a moment to the consideration of an important intangible asset of the Association, namely the good will that it enjoys throughout the United States and many foreign countries where the book of methods of the Association is used daily by chemists and other scientific workers in the analytical field. In the words of Charles Fletcher Dole: "Good will is the mightiest practical force in the universe." The latent good will that exists for the Association is based on the respect shown for its methods by our professional associates, who know of the extensive collaborative work conducted by our members before the Association sets its seal of approval on a method by designating it "official."

The Association's chief functions are to develop methods of analysis, to test such methods by collaborative work, and to adopt only those methods that have been shown to be workable and accurate in the hands of the average skilled analyst. By actual laboratory trial, this procedure raises each chemical, microchemical, or biological analysis from the plane of a "one-man method" to a point where many competent chemists, microanalysts, and bacteriologists can analyze samples of unknown composition and obtain similar accurate results. That such work is essential is well recognized. Who is there among us that has not at some time attempted to apply a "one-man method" to a sample, and before proceeding very far has found an involved program of analytical research comprising modifications, sometimes quite extensive, of published procedure and of apparatus. While the testing of analytical methods cooperatively is one of the most important duties of the Association and the one that is perhaps the most widely recognized, other objectives that are set forth in the constitution of the Association include the following: To secure uniformity in the statement of analytical results; to conduct, promote, and encourage research

<sup>1</sup> Presented before the 58th Meeting of the Association of Official Agricultural Chemists, held at the Statler Hotel, Washington, D. C., October 27, 1943.

in chemistry in its relation to agriculture; and to afford opportunity for the discussion of matters of interest to agricultural chemists.

The active membership of the Association is restricted to scientific members of official agencies of North America having to do with the analysis, research, and control of food, grain, and stock feeds, drugs, soils, fertilizers, agricultural liming materials, insecticides, fungicides, caustic poisons, naval stores, paints, leathers, and tanning materials. These workers hold membership in the Association by virtue of their official positions; when they sever their relations with their official institutions, they automatically lose such membership.

The methods published by the Association, "Official and Tentative Methods of Analysis of the A. O. A. C.," is the "bible" of nearly all food analysts in the United States and in many foreign countries. At public hearings on standards for food and in State and Federal regulatory actions that have progressed to a point where they are the subject of court controversy, the methods of analysis given in this book are respected and are usually accepted without question. Moreover, many Federal standards and specifications for foods contain a specific clause to the effect that chemical analyses shall be made in accordance with methods of the A. O. A. C.

The good will engendered by the Association's method of doing business and by leaders such as Johnson, Babcock, Wiley, B. B. Ross, Frear, Ladd, and a host of other less well-known officers of the Association, is a precious asset, and one that our members are obligated to preserve. This objective can be accomplished only by approaching our tasks with a new zest and a determination to make the collaborative testing of published methods and the devising of new methods keep pace with the rapid developments of the times. Some of these developments that relate to war-time foods will be considered here, and an attempt will be made to point out some of the problems of our Association that should be dealt with in the immediate future.

Among the noteworthy aspects of war-time foods that may be mentioned are the expansion of the dehydrated and frozen food industries, the large increase in the kinds and quantities of vitamin-enriched and vitamin-fortified foods, the development of the basic and operational rations of our armed forces for use at home and overseas, and the production of the so-called "extenders" or food substitutes to meet war-time scarcities and civilian needs.

#### DEHYDRATED AND FROZEN FOODS

Commercial dehydration, which in the case of egg drying in the United States dates back to the Civil War, obtained its real start during the Boer War and World War I, when food had to be sent long distances and cargo space was limited. It received a tremendous impetus in the early months of the present war owing to the same necessity of conserving space in



overseas shipments and to the shortage of tin supplies from Far Eastern tin-producing areas now under Japanese control. In normal times the water content of dried fruits usually ranges between 20 and 25 per cent, and that of dried vegetables between 10 and 15 per cent, whereas dried fruits are now dehydrated to less than 1 per cent moisture, and properly dehydrated vegetables generally contain less than 7 per cent and sometimes as low as  $3\frac{1}{2}$  per cent. The advantages in favor of the dehydrated products from the standpoint of shipping space are obvious.

As of June, 1943, the War Food Administration reported that there are 1626 food dehydration plants in operation or under construction in the United States, including 1107 roller-process dried skim milk plants, 204 spray-process dried skim milk plants, 13 meat plants, 126 egg drying plants, and 176 vegetable dehydration plants. As of January, 1943, the estimated requirement of dehydrated food for allied shipment in 1943-44 amounted to approximately 845 million pounds and included mainly dried milk and skim milk, eggs, white potatoes, cabbage, carrots, beets, rutabagas, tomato products, baked beans, and pork. It is estimated that 300 million pounds of fruits and vegetables will be dehydrated this year compared with 25 million pounds several years ago.

Water can be taken out of butter and the butterfat so treated that it will not only take 20 per cent less shipping space than butter but will keep almost indefinitely without refrigeration. Dehydrated pork made from precooked lean pork is being substituted for fresh meat. Dehydrated soup mixtures made of pea meal, soy bean grits, and dry skim milk, with a protein content of approximately 35 per cent, are being manufactured to the extent of millions of pounds. Also a dehydrated vegetable stew mixture is made in powder form from precooked beans, peas, soy bean grits, whole barley, and other grains, dehydrated diced vegetables, brewer's yeast, and seasoning. By a new method for dehydrating American cheddar cheese reported during the year, the moisture content is reduced from 36 per cent to about 3 per cent. The resulting product contains all the fat and other solids of the original cheese. Dehydrated apples in a form known as apple nuggets are used to prepare apple sauce and apple pie. The Army has a specification for so-called tomato juice cocktail, dehydrated in flaked and powdered forms and prepared from tomatoes or tomato purée, sugar, salt, stabilizers, spices, and seasonings, with a maximum moisture content of 5 per cent. These are a few of the "oddities" in the dehydrated food field.

High frequency heating, which is playing its part in the development of improved methods for the dehydration of food, utilizes the phenomenon that imperfect dielectric materials will absorb electric energy in the form of heat when placed in an electric field. One process based on this principle is stated to reduce the moisture content of dehydrated vegetables in brick form to 1 per cent.

A recent development in the food field is the compression of dehydrated

foods such as soup in brick form and practically free from air. Dehydration removes most of the water from a food, but compression goes one step further, squeezing the dehydrated food into the smallest size practicable and thereby saving enormous amounts of shipping and storage space. In its simplest form moderate compression is accomplished by tamping, vibrating, and compacting a product so that waste space in a container is held to a minimum. Flour for example can be so compacted that approximately 20 per cent more will go into an ordinary sack. Among the foods that have been successfully compressed for packaging are flour, oatmeal, many dehydrated or dried vegetables including carrots, beets, cabbage, and sauerkraut, "beet and potato hash," fruits and fruit juices, eggs, and milk. The finished blocks are protected against deterioration in color, flavor, and nutritive value by covering them with various sorts of moisture-proof materials. Cardboard, parchment, foils, waxed linings, cellophane, flexible plastics, and many other materials are being tested for this purpose. To ascertain the best methods of dehydration and the most suitable forms of modern equipment for dehydrating fruits and vegetables, research workers are conducting extensive studies and investigations on rehydration of dried fruits and vegetables, on control of fat deterioration and enzymatic actions, on the destruction of vitamins in the various stages of manufacturing, on the retention of vitamins under different conditions of storage and shipment, on the influence of moisture on keeping quality and on the suitability of various types of available materials for containers. Necessarily, much of this research is dependent on suitable methods of analysis.

Exclusive of frozen meats, eggs, cream, uneviscerated poultry, and bulk fish, the frozen food industry has expanded in the United States from a production of 556 million pounds in 1940 to an estimated production in 1942 of 824 million pounds. Twelve different types of freezing methods are in use. As in the case of dehydrated foods, the expansion of our armed forces, the necessity of conserving storage and shipping space, and the shortage of tin are controlling factors in this increased production of frozen foods. A ton of beef carcasses occupies 100 cubic feet; after it has been boned and packed into containers for freezing it occupies only 35 cubic feet. The frozen food industry is therefore playing an important part in the war effort. In the post-war period, unquestionably, quality factors of frozen foods including color, flavor, physical defects, and maturity, will be given even greater attention.

#### ENRICHMENT AND FORTIFICATION OF FOOD

The addition to food of vitamins and minerals on the basis of dietary deficiencies in significant population groups has been greatly accelerated by conditions produced by the war. Attention was focused on this subject by the fortification of ersatz foods by the Germans during the early days of the war, and in 1940 the movement for this fortification of foods re-

ceived its greatest impetus. Enrichment of all white bread is required under Food Distribution Order No. 1 of the War Food Administration, effective January 18, 1943. Under this order the term "enriched" means that the bread shall be made from enriched flour containing the ingredients in the quantities required by the regulations under the Food, Drug, and Cosmetic Act, or that equivalent ingredients shall be added to plain flour during the mixing of the dough.

Under the Federal Food, Drug, and Cosmetic Act, standards of identity have been promulgated for a number of foods that either require or permit the addition of vitamins or minerals.

A standard for oleomargarine announced in June, 1941, permits, with label declaration, the addition of a minimum amount of vitamin A. The vitamin A is added as fish liver oil or as a concentrate from such oil, with any accompanying vitamin D and with or without added vitamin D concentrate. Practically all the margarine now being sold in the United States has vitamin A added.

Proposed standards for enriched bread and enriched rolls or buns, published in August, 1943, require within specified limits the addition of thiamin, riboflavin, niacin or niacin amide, and iron, and permit the addition within specified limits of vitamin D and calcium. Standards for enriched flour, enriched bromated flour, enriched self-rising flour, and enriched farina were promulgated in May, 1941, and with the exception of enriched farina, all were amended in July, 1943. It has been announced that a supplemental order will issue with respect to the proposal for an amended definition and standard of identity for enriched farina. The standards and amended standards for enriched flour and enriched bromated flour require the addition, within certain limits, of thiamin, riboflavin, niacin or niacin amide, and iron, and permit within certain limits the addition of vitamin D and calcium.

The amended standards for enriched self-rising flours are similar to those just mentioned with respect to enrichment factors except that calcium is a required instead of an optional ingredient. The standard for enriched farina requires certain minimum amounts of thiamin, riboflavin, nicotinic acid or nicotinic acid amide, and iron, and permits the addition of minimum amounts of vitamin D and calcium.

Standards for evaporated milk and concentrated milk promulgated in July, 1940, permit, with label declaration, a minimum quantity of vitamin D and any vitamin A that may accompany vitamin D when the latter is added in the form of a concentrate obtained from natural sources. Evaporated milk with added vitamin D has been available for a number of years, the first brand appearing on the market in 1931.

An informative statement of policy regarding the addition to foods of nutritive ingredients was issued in July, 1943, by the Federal Security Agency and reads in part as follows:

Because of the lack of adequate production of a number of foods high in certain nutrients and the lack of consumer knowledge of nutrition, appropriate enrichment of a few foods widely consumed by the population in general or by significant population groups will contribute substantially to the nutritional welfare of consumers and to meeting their expectations of benefit. Enrichment of those foods which are not a substantial part of the dietary of any significant group tends to confuse and mislead consumers through giving rise to conflicting claims of nutritional values and by creating an exaggerated impression of the benefits to be derived from the consumption of such foods.

If the customary process of manufacturing a staple food refines it so as to remove significant quantities of nutritive factors present in the natural product from which the food is made, and if the refined food is a suitable and efficient carrier of the factors so removed, some nutritionists advocate the restoration of such factors to the levels of the natural product as the most desirable basis of enrichment. To the extent that restoration serves to correct deficiencies of such factors, it is consistent with the promotion of honesty and fair dealing that refined foods be enriched on a restoration basis. However, when the evidence shows that the restoration levels are too low to correct deficiencies, or that deficiencies exist in other factors for which the refined food is an efficient carrier, the promotion of honesty and fair dealing may require the inclusion of corrective quantities of nutritive factors in the enriched food even though such factors are present in smaller quantities or wholly lacking in the natural product from which the food is made. Similar considerations may require the enrichment of unrefined foods.

In addition to the specific regulations dealing with enriched foods under the Federal Food, Drug, and Cosmetic Act, a number of tentative Army specifications provide for the purchase of enriched foods. At this time such foods include flour, dehydrated soup mixes, yeast, certain army field rations, and lemon juice powder.

Flour purchased for the armed forces of the United States is enriched in conformity with the requirements of the Federal Food, Drug, and Cosmetic Act. The tentative specification (C. Q. D. No. 26 A, July 30, 1942) of the Quartermaster Corps for dehydrated powdered soup mixtures and pastes (vegetable soup, yellow pea soup, green pea soup and bean soup) requires the addition of a minimum amount of vitamin B complex concentrate consisting of a dehydrated extract of brewers' yeast. Army field ration D, a specially compounded 4-ounce chocolate bar, contains a minimum amount of added thiamin hydrochloride. The Army's enriched yeast contains a minimum quantity of thiamin and may contain other vitamin and mineral additions acceptable in good commercial practice. The Army's "lemon juice powder synthetic" contains a minimum amount of ascorbic acid. In August, 1943, the Food Distribution Administration issued an invitation for bids for a 2-ounce chocolate bar for export to contain a minimum proportion of thiamin hydrochloride corresponding to that in Army field ration D. The government requirements for enriched foods cited will serve to illustrate current trends in the national nutrition program. It appears that the influence of food faddists is lessening and that future development of enriched foods will be on a sane and practical basis.

Among war-time foods there are the famous Army field ration D and the other Army emergency field rations including C, K, and J, the latter being a jungle ration. Field ration D consists of three 4-ounce chocolate bars specially compounded so that they will not soften unduly at even desert or tropical temperature and reinforced with thiamin chloride to help metabolize their calories. This is a special product with no commercial counterpart. Field ration K, supper unit, consists of the following components: Two packages of biscuits of two types, 1 can of meat or cheese, a 2-ounce bar of sweet chocolate fortified with thiamin hydrochloride, bouillon powder, 1 stick of chewing gum, and 4 cigarettes. It is of interest that a captured Japanese emergency ration was found to consist of 3 cakes of pressed fish and bread, 1 cake of compressed pickled plums, 3 cakes of pressed sugar, and 3 packages containing 7 cakes each of pressed whole wheat. The size of the Japanese compressed ration was 28 cubic inches. The components of each serving unit of ration K are placed in a carton having a capacity of 31.6 cubic inches. This combat ration was developed as a result of a request from parachute troops for an extremely light and compact ration.

#### ERSATZ FOODS

The manufacture and distribution of so-called "extenders," such as "coffee extenders," "chocolate extenders," "pepper extenders," "butter extenders," and the like, have resulted from a scarcity of genuine food ingredients due to war conditions. Many of these products are such poor imitations of the genuine that once tried they are discarded and never repurchased by the same individual. It is reported in the food industry press that these so-called food extenders, "which offered a one-way pull out of grocers' shelves" when rationing first began, have lost their sales magnetism and remain stationary in warehouses. However, the role played by food products that vary so greatly in composition and use as do the food substitutes now on the market cannot be summed up in so general a statement and they must continue to be subjected to close scrutiny by food control chemists, and methods for their examination should be developed when necessary. These products are of little concern to the Army and Navy and to officials in charge of exports of food to our Allies, as their supplies are purchased under specifications calling for genuine foods and genuine normal components.

These products are also known to the industry under such terms as food substitutes, boosters, stretchers, extenders, fillers, and ersatz foods, and some of them were well-known adulterants of food in the early days before the enactment of Federal and State food laws. In many instances their use ceased to be classed as adulteration under the food laws when the labels were appropriately revised to indicate their true composition. Now we have them with us again in greatly increased varieties and numbers. There is hardly a type of food for which a "booster" has not been devised due to a scarcity of the genuine food coupled with a persistent demand

on the part of the public for the articles of food to which it has become accustomed. Conditions too numerous to discuss fully contribute to such scarcity, but a few might be enumerated. Some spices are unavailable now or available in much less than their usual quantities because the areas of their production have been invaded by the Axis powers. The shipment of cacao beans from Africa was curtailed by enemy activity on the high seas and supplies of pectin and citric acid for domestic use decreased because of an increase of exports to our Allies, the needs of our own armed forces for lemon juice and jams and jellies made with these ingredients, and the restrictions on metals needed to expand manufacturing equipment. These and many other diminishing supplies of our accustomed foods have made a field day for ingenious manufacturers of substitutes, many of which may serve a useful purpose. On the other hand, many of them are undoubtedly on their way out because of their general worthlessness. They are seldom harmful to health, but they may be insanitary, especially when they consist of by-products of food manufacture that are normally used for fertilizer, or burned or otherwise destroyed. Frequently, food officials are importuned to relax their enforcement of food laws with respect to standards and permit the sale of these more or less debased foods, the nutritive value of which is usually less than that of the genuine articles they are intended to replace. Frequently the labeling leaves much to be desired, as it implies that these foods will in every way take the place of the genuine articles, which is seldom the case.

Let us consider the composition of some of these ersatz foods with a view eventually to determining the adequacy of our analytical methods with respect to the detection and determination of these substitute ingredients, some of which are new and others of which are present in new combinations.

Chocolate boosters are rampant. They are sold to proprietors of dairies, bakeries, and other food establishments for admixture with the curtailed supply of chocolate or cocoa to permit normal output of the finished chocolate milk, chocolate cake, or other chocolate product. Basic raw materials used in their manufacture are ground, roasted soya beans, roasted cottonseed flour, cocoa shells, chicory, caramel, and imitation chocolate flavoring. When in liquid form they also contain a solvent, a sweetening ingredient, and frequently benzoate of soda to preserve them.

Coffee stretchers appear to be made of anything that is readily available, and they include roasted malt, rye, barley, soy beans, chick peas, wheat, spent coffee grounds, and chicory. The cereals are roasted and sold in ground form for mixing with coffee for household use; extractions of them are also sold for mixing with beverage coffee. Government inspectors have found coffee mixed with cereal in proportions as high as one part of cereal to three parts of coffee and with no such indication on the package. Ordinary dried lawn grass has been palmed off as tea.

Substitution in the field of dairy products consists of decreasing the

fat content of cheese and ice cream, which is likely to increase the proportion of water with resultant lowering of nutritional value. So-called butter stretchers consist of processed butter or synthetic flavorings or both, together with a vehicle or solvent. Diacetyl, butyric acid, and butyric acid esters are widely used in these flavorings. Large quantities of milk and some butter, together with a stabilizing agent, are combined to produce an article simulating butter. A so-called Victory spread of this character contains only 70 per cent butterfat.

Suet and other fat of the type used in the production of soap and glycerol are accumulated as meat trimmings and rendered for sale as edible fats. Up to a half of normal shortenings is replaced by the mono- and diglycerides of fat-forming fatty acids and by similar products in which propylene glycol takes the place of glycerol.

A so-called egg substitute was found to consist merely of yellow colored corn starch. Other such substitutes made from flour, starch, sugar, and some egg are sold to bakers and other food manufacturers. Flour is substituted in part for egg albumen. In the essential oil field we find imitation oils of cassia, bergamot, cinnamon, citronella, geranium, lavender, lemon grass, peppermint, and sassafras. Among these essential oil substitutes are imitation anise oil consisting of synthetic anethol, imitation allspice oil made from eugenol and sesquiterpenes, imitation cassia and cinnamon oils made from cinnamic aldehyde and small amounts of esters of cinnamic alcohol, and imitation coriander oil made from linalool. No need has arisen for substitute lemon or orange oils, as the genuine oils are plentiful in the United States. However, chemists are prepared to produce imitations for such oils, if necessary. Spice substitutes are legion. Frequently, these consist of exhausted spices, or cereal products impregnated with flavoring material to simulate spices. They include a cinnamon substitute made from ground pecan shells flavored with cinnamic aldehyde, and another made from a dry ground cereal processed base with oil of cassia, cinnamic aldehyde, and other aldehydes and esters. So-called caraway seed has been found to consist of dill seed flavored with oil of caraway. Imitation white pepper, cinnamon, nutmeg, and paprika consisting of 20-50 per cent of added starch and in some cases, artificial color, have been encountered. A nutmeg substitute consists of ground soya bean and oil of nutmeg.

The chicle in the popular confection chewing gum is replaced in whole or in part with the diglycerides, and esters of propylene glycol.

In the field of bakery goods a processed vegetable pulp artificially colored yellow and flavored with imitation pineapple flavor has been proposed as a substitute for crushed pineapple, and crushed peaches artificially colored red, with added raspberry seeds, have been substituted for raspberries. Raspberry and blackberry seeds are obtained as a by-product in the manufacture of seedless raspberry and blackberry jams and in the form of pomace in the manufacture of jelly juice.

Dehydrated unpeeled bananas, sugar, and stabilizer have been substi-

tuted for raisins. A cinnamon roll dusting powder was found to consist of exhausted ginger, rye flour, corn sugar flavored and colored with cinnamic aldehyde, and caramel. Maleic acid, adipic acid, hydroxy-acetic acid, and saccharic acid have been proposed or actually used as food acidifiers. Some of these products are unfit for food because of their toxicity. Incidentally, maleic acid should not be confused with malic acid, which is a recognized food acidifier. Phosphoric acid and acid phosphate have come into greatly increased use because of the scarcity of the common organic acids. Several emulsifying agents and wetting agents have been proposed for use in food, including some of the alkyl aryl sulfonates, propylene glycol monostearate, and ursolic acid, which is recovered from cranberry waste. Sodium alkyl aryl sulfonate is a detergent that is used in fruit washing. Ethyl stearate has been considered as a substitute for cocoa butter. Various glycerol substitutes have been encountered, including mixtures of diethylene glycol and corn sirup, and various lactates and salts with corn sirup.

Among the unusual or non-permitted preservatives newly proposed or actually being used are monochloroacetic acid, dichloroacetic acid, a salt of propionic acid, and the old-time adversaries of food officials—salicylic acid, boric acid, and peroxides of calcium and of hydrogen.

Among the so-called meat extenders, which are intended to be mixed with meat as in the preparation of sausage or meat loaf, we find processed soy beans and other legumes, with or without dried skim milk.

Coconut substitutes consist of small white corn flakes with vegetable oil and imitation coconut flavor. Pumpkin seeds have been substituted for nut meats in packages of salted nuts and puffed rice for peanuts in peanut bars. Another nut substitute is a mixture of malted kernels of wheat and corn, malted barley, corn sugar, and salt. Sodium alginate or gelatin, which have their own legitimate fields, are being used in place of pectin. Mineral oil in place of vegetable oil has been found in salad dressings, and liquid paraffin has been used instead of vegetable oils for baking. A so-called sugar substitute, which would not serve at all as a substitute, was found to be merely a sugar-inverting agent consisting of tartaric acid, Indian gum, soda, salt, and dextrin. A "honey stretcher" consists of 20 per cent honey and 80 per cent invert sugar sirup. Saccharin has been found recently in soft drinks and other foods in partial substitution for sugar.

Few, indeed, of the food constituents that have been enumerated are either new or unique; many of them are being used in new combinations and forms resembling foods to which the public has become accustomed. Frequently, even fully informative labeling does not apprise the buyers of the composition of these foods since they are used by manufacturers as raw materials for other food that may not be labeled, as for example imitation chocolate consisting of cocoa shells, which is used in the preparation of so-called chocolate cake.



## WAR-TIME PACKAGING OF FOOD

Any consideration of war-time foods would be incomplete without some reference to the containers used for such foods. The necessity of packaging enormous quantities of old and new types of foods for overseas shipments under the restrictions that exist on available container materials has resulted in the development of many new types of food containers and their use for both foreign and domestic shipments.

Among the kinds of war packaging used are containers made of electrolytic tin plate, bonderized plate, and deoxidized steel, flexible packaging films and "surf tested" cases and ordinary containers made with special linings and closures, or with as little glass as possible. Electrolytic tin plate saves tin in that it carries only about 0.5 pound of tin per base box instead of the usual 1.5 pounds and owing to the method of uniform application, it is said to afford greater protection than would be expected by the lesser amount of tin. In one type of electrolytic coating, the tin is deposited from an acid solution of stannous sulfate containing sulfonic acids, gelatin, and other components, and in another type from an alkaline bath of sodium stannate. For protection against corrosion, such lightly coated plate is enameled on the inside of the can for domestic purposes, and for overseas shipments it is enameled on the outside also.

Bonderized plate is similar to the base plate of the tin can but is chemically treated to reduce the rate of corrosion. In this process steel sheets are treated with a solution of zinc dihydrogen phosphate containing an oxidizing agent and a catalyst, which results in coating the steel surface with crystals of zinc and ferric phosphates. The bonderized surface is given at least one coat of organic enamel. The increased demands for organic coatings for electrolytic tin plate and bonderized steel have been met by the use of dehydrated castor oil and a number of domestically produced synthetic resins, which replace in whole or in part tung and perilla oils and some of the fossil resins that are now unobtainable.

Another war-time innovation, due to scarcity of tin supplies, is the proposal to substitute silver for tin as a container lining. It is said to be practicable to manufacture relatively low-cost silver-lined containers such as barrels, drums, and cans because they have the advantage of resistance to alkalis, organic acids, and certain concentrations of hydrochloric and other mineral acids. This development is still in an experimental stage. Deoxidized steel is enameled on both sides and is used for steel drums, ends of fiber cans, and for packing dry products. Flexible packaging films have been developed as a result of the requirements of the Army Quartermaster Corps for overseas shipments of food and other commodities in the face of restrictions on usual container materials such as tin plate, black plate, rubber, and aluminum foil. They consist of a base of fiber or cellulose products coated with films of modified waxes, nitrocellulose, resins, varnishes, lacquers, and other materials. Two or more sheets may be combined to produce a "laminated" film, which may be again coated.

These containers are resistant under climatic and transportation conditions that would destroy the old type containers. Numerous packaging and shipping tests to determine the efficiency of packaging films have been devised.

The effect of the war effort on glass packaging has been the speeding up of that industry's program, which has been in effect over a period of years, of reducing the weight of glass containers, standardizing their sizes and shapes, and mechanizing the packaging lines. The use of the smaller sized caps for the plastic, wax-lined, glass containers has increased owing to shortages of plasticized vinyl copolymer, rubber hydrochloride, and chlorinated rubber hydrochloride-coated paper materials, normally used for lining such closures.

#### FILTH IN FOOD

With this brief glimpse of war-time food I shall return for a moment to the basic function of the Association in improving and testing methods of analysis because necessarily it follows that our methods of food analysis should be reviewed in the light of present-day sophistication of food and the use of new types of containers, and revised and expanded where necessary, particularly in regard to the detection and quantitative determination of filth. A number of the substitute ingredients being used are normally by-products of food manufacture and heretofore have been generally regarded as refuse material fit only to be burned or dumped. The conditions under which such by-products are produced, stored, packaged, and transported frequently result in insect and rodent infestation and the incorporation of filth in other forms. Consequently, methods for the detection and determination of filth in food are becoming increasingly important. Closely allied to filth is decomposition, methods for which are needed in the case of eggs, dairy products, fish, fruit, and other food. Federal food chemists are successfully attacking these problems, and the results of their efforts will be reflected eventually in our methods.

#### REVISION OF METHODS OF ANALYSIS, A.O.A.C.

Chapters in our *Methods of Analysis, A.O.A.C.* that seem to need particular attention are those on soils, sugars, vitamins, oils and fats, and those containing microanalytical and microchemical methods. One of our correspondents stated recently with respect to the revision of the soils chapter:

In the field of soils all of the outmoded methods have not yet been replaced by methods for characterizing quantitatively the properties of soils and soil colloids which have a rapidly growing significance. Many of the published papers from this and other laboratories in recent years have been on the subject of methods. This was necessary as the whole field was in a state of flux, but the time is about ripe now for a systematic and thorough study of these methods, such as only the A.O.A.C. can do.

Improved methods of analysis should be tested and incorporated in the

chapter on sugars and sugar products, especially those that are specific for particular sugars or classes of sugars,—for example, the determination of dextrose in mixtures of dextrose and other sugars and distinguishing between monosaccharides and other classes of sugars. The food analyst frequently encounters such mixtures as acid-hydrolyzed starch products and malt products containing varying amounts and kinds of sugars, for the analysis of which the A.O.A.C. methods are not wholly adequate. A method that distinguishes between monosaccharides and disaccharides or other high molecular-weight sugars is available for collaborative study. Some of the more rapid fermentation methods should be given consideration in determining the constituents of sugar mixtures. Although many strains of yeasts that have been developed are specific for various sugars, methods of sugar analysis making use of them are generally too time-consuming to be serviceable in law enforcement work.

The separation of various sugars by the preparation and subsequent distillation of their esters and ethereal derivatives is worthy of study and there is a definite need for better methods for the determination of dextrans used in the analysis of acid conversion starch products, malt products, confectionery, and the like. Methods now available are usually subject to errors owing to contamination with other materials, or they are based on determinations that are "elastic," such as polarization, in that starches from various sources differ greatly in specific rotation, and no doubt their dextrans are subject to similar variations.

It has been shown recently that nine reprecipitations of dextrans from corn sirup are necessary before their reducing powers and specific rotations are constant, thus indicating absence of occluded material. Dextrans that are precipitated under the same conditions from corn sirup of different degrees of conversion have been shown to possess different specific rotations. Obviously, the procedure for the determination of dextrin in food needs to be thoroughly investigated. Methods that are more satisfactory than A.O.A.C. methods for the determination of moisture in sugars by drying and distillation are available and should be studied.

Application of recently acquired information concerning the use of vitamins and methods for their manufacture has been more rapid than the development of satisfactory methods of assay. An example of this condition was the promulgation of a definition and standard for enriched flour under the Food, Drug, and Cosmetic Act in May, 1941, requiring nicotinic acid as an ingredient. The hearings for taking the testimony upon which this standard was based were held in the fall of 1940, and at that time there were no methods available for the quantitative determination of nicotinic acid.

There are two methods for the assay of vitamins in the *Book of Methods*, namely, Vitamin D Milk and Vitamin D for Poultry, both of which are tentative. In addition, the following tentative methods have been adopted

and published in our *Journal*: Biological assays for vitamins B<sub>1</sub> and K, and microbiological assay for riboflavin. It appears that the commonly used methods for the determination of vitamins A and D are those prescribed by the U. S. Pharmacopoeia nine years ago. These methods are sufficiently well known so that they need no special consideration. However, there is need for the adoption of methods for all the other vitamins known to be necessary in human nutrition, viz., ascorbic acid, thiamine, riboflavin, nicotinic acid, and vitamin K. Real advances have been made in developing methods for these vitamins during the past two or three years and they should be tested cooperatively and passed on by the Association at an early date for incorporation into the next edition of *Methods of Analysis*.

The chapter on oils, fats, and waxes, while containing adequate methods for the determination of fatty acids, is somewhat deficient in methods for detecting rancidity and other forms of deterioration. The need for tests for quality, such as the peroxide number, will become more urgent as quality standards are developed and as requirements controlling quality are incorporated into specifications. Available rapid methods, such as the refractometric method of determining the fat content of flax seed, should be studied and their application extended to other products, and new rapid and accurate methods should be devised to facilitate analyses made in connection with regulatory operations where speed of examination, without sacrifice of accuracy, is an important factor.

A method for the microanalytical examination of tomato pulp, purée, sauce, and paste is contained in the chapter on vegetables and vegetable products. This one method constitutes the only microanalytical procedure in the *Book of Methods*. Obviously, then, this chapter is weak in so far as microanalytical procedures are concerned, and it should be substantially expanded in this particular. While the method given is of value to regulatory officials, it covers only one type of vegetable, tomatoes, and regulatory officials are daily encountering many other varieties of vegetables and vegetable products in need of regulatory control and for which there are no official methods of analysis.

The examination of food products for filth constitutes an important part of regulatory food programs, and the need for numerous methods for the examination of a wide variety of products is essential if the *Book of Methods* is to be adequate. Microanalytical methods are needed for the examination of cereals and cereal products, flour, and meal products; for confectionery, sugars, and sirups; for dairy products, fish, spices; for fruits and fruit products; and for a variety of vegetables and vegetable products. Many such methods have been correlated and compiled for the use of regulatory officials by the Food and Drug Administration, and it is hoped that gradually such methods will be made available for consideration for adoption by the Association.

In the chapter on drugs, the microchemical tests listed for alkaloids should be expanded. Optical constants are not included in the identifications of crystalline drugs in U.S.P. XII, although optical crystallographic data are exceptionally valuable in determining the identity of certain crystalline drugs. The portion of the chapter on drugs dealing with microchemical tests could be improved immeasurably by the addition of crystallographic data on the drugs listed in U.S.P. XII. Such data are available in the files of the Food and Drug Administration.

*Methods of Analysis*, A.O.A.C. contains numerous procedures for the determination of the same constituent in different products. In many instances such procedures vary in details that could be made identical, except for preparation of sample, without affecting the accuracy or time of the determination. For example, drying temperatures for moisture determination are given as the temperature of boiling water in one method, 100°C. in an air oven in another, and 105°–110°C. in another method. Specific gravity is determined at 20°/20°C. in one method and at 15.5°/15.5°C. in another. In one method the procedure for determining specific gravity is described in detail, and in another method general terms are used without reference to the detailed procedure. The methods should be scrutinized from this point of view, particularly those for moisture, ash, acids, alcohol, arsenic, calcium, carbon dioxide, chlorine, fat, nitrogen, phosphoric acid, solids, specific gravity, starch, and sulfur. Not only will space in the book be saved by making the procedures uniform wherever practicable, but the analyst will not be led to place undue emphasis on procedural differences that are actually of no significance.

Methods that depend on the instruments of precision used for research and testing should be reviewed to determine whether provision should be made for the utilization of all improvements made in such instruments and of instruments that were unknown when the methods were promulgated. Great advances along these lines have been made by instrument makers.

The field of activity of the A.O.A.C. is very broad. Only a few of our problems have been referred to here, but they are sufficient to illustrate the importance and urgency of our tasks. The intrusion of the war has of necessity greatly reduced the time and effort that can be given to the basic work of the A.O.A.C., but such time as is available or can be made available without sacrificing war effort should be expended only after an unusual degree of thoughtful study, with a view to selection of the most pressing and important subjects for investigation. When that longed-for day that signals the end of the war arrives, we shall have many loose ends to gather up. Let us look forward to that time with renewed enthusiasm for the work of the Association.

## ORDER OF PUBLICATION

The reports of the committees presented on the last day of the annual meetings are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees, and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

## SECOND DAY

### THURSDAY—AFTERNOON SESSION

#### REPORT OF EDITORIAL BOARD

By W. W. SKINNER, *Chairman*

I shall make only a brief statement about the work of the Editorial Board; the detailed statement for *The Journal* will be made later by Mr. Lepper.

The editorial work has gone along on the plan that has been in effect for some years. We have some problems, one particularly about *Methods of Analysis*, A.O.A.C. (familiarily called the *Book of Methods*), because the next revision is due in 1945. The supply of copies of this publication is nearly exhausted. To meet the demand until 1945 there remain only about 450 copies out of the 7,000 copies of the original edition. There has been a continued and gratifying sale of this book, even during this war period, in fact it really seems that sales have been stimulated by the war. The *Book of Methods* is a unique volume, and we have all taken a part in its production. I shall repeat the statement that I have made previously that in certain types of court procedure the three books that are accepted are the Bible, our standard of morals; the Pharmacopoeia, our standard for drugs; and the *Book of Methods*, our standard for determining the composition of material products. This is a very high position. The *Book of Methods* is one of the most widely distributed books printed in the English language. It is quite surprising to note from the list of purchasers that this book goes to the four corners of the earth.

We have had several offers to publish *Methods of Analysis* in other than the English language, particularly in Spanish. Two years ago a proposition was made to us by an agricultural association in Cuba to have the book translated into Spanish. There would be some advantage in doing so, and as a part of the good-neighbor policy we gave it serious thought, but the people who were interested did not seem to have the financial and other backing necessary. It would be unfortunate—if the book is translated—to permit it to be done in a way that would detract from the credit that we have already attained in the public mind. More recently there have been two other offers made to translate the book into Spanish. One of

these was of a commercial nature, while the other was from an agricultural college. Fourteen chapters, the chapter on definitions, and the index had already been translated and specimen pages were submitted by the latter organization. The new officers should consider this matter during the coming year. I do not know what the Association will do if the supply of *Methods of Analysis* is exhausted this year. The new edition will not be available until the latter part of 1945. A reprint of the present edition is out of the question, and I do not believe it would be advisable to advance the date of the revision, but that of course must be determined in the future by the Board of Editors.

Approved.

## REPORT OF EDITORIAL COMMITTEE OF *THE JOURNAL*

HENRY A. LEPPER, *Editor and Chairman*

As may be noted in Dr. Skinner's report as Treasurer, *The Journal* has returned a small profit. It has never been expected to be a source of revenue, but it is always gratifying when there is not a deficit.

Several years ago the policy on reprints was liberalized because the Association was considered to be in a position to offer reprints to authors of contributed papers—50 without cost. This is quite an item of expense to *The Journal*. Shortly after that policy had been inaugurated it was pointed out that very often a referee report is in the same classification as a contributed paper; that is, it is the result of individual research, not simply a report of collaborative study of some method. Therefore the Executive Committee authorized the Editorial Board to follow the same policy in regard to referee reports whenever it was considered desirable to do so. This plan has worked out very satisfactorily, except that repetition in printing the details of methods presented a problem in some instances. Often the referee report includes a long method that has been adopted, and printed in the first number of *The Journal* following the meeting, where the changes in methods are officially announced. These changes become effective, according to the by-laws, 30 days after such publication of the details of directions. The referee report follows in a later number, and to be complete in reprint form requires a repetition of the details of the method. Provisions for reprints at the time of the first printing can probably be made if the number to be needed is known. The Editorial Office may be able to work out satisfactory arrangements in such cases if the length of the directions makes the saving of paper of importance.

A year ago *The Journal* reporting the 1941 meeting ran 971 pages, only a few below Volume 24 for the 1940 meeting, which had over 1,000 pages, the largest volume in any one year. In the absence of the regular annual

meeting of the Association last year, *The Journal* ran 584 pages. A substantial number of reports were ready for presentation at the meeting, and these were published in Volume 26. This year it is realized that *The Journal* may be smaller owing to the number of referees now in the armed forces. *The Journal* will continue to publish contributed papers and those that deal with our work are solicited.

It may be recalled that in 1931 an index of all the proceedings since the organization of the Association was published. This year a second index, to include the proceedings from 1931 to 1940, was compiled and published. This index will be distributed to the current subscribers of *The Journal*. We have no way of distributing it to all the subscribers of the 10 year period, but extra copies will be available for purchase at one dollar a copy to those who no longer subscribe but who may desire the ten-year index.

The work of the committee has been directed to promote the revision of *Methods of Analysis* (the 6th edition). The revisions come at five-year periods, and so the next edition should be ready for distribution in 1945. However, if it seems to be inadvisable or impossible to have a meeting of the Association next year because of war conditions, it might not then be desirable to revise the *Book of Methods*. Certainly it would not be advisable to issue an edition that does not actually constitute a thorough revision of the methods. A meeting next year will make it possible to issue a revision in 1945 up to the usual progressive standard.

Approved.

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No report of the Board of Editors, *Methods of Analysis*, A.O.A.C., was given by the Chairman, E. M. Bailey. For remarks concerning the 6th edition of this publication, see the two preceding reports.

No report was given by the Committee on Quartz-Plate Standardization and Normal Weight.

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## REPORT OF COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATIONS OF RESULTS ON FERTILIZERS

### *Official, Final Action* NITRATE OF SODA AND POTASH

*Nitrate of soda and potash* is a commercial product containing nitrates of sodium and potassium, and it shall contain not less than fourteen per cent (14%) of nitrogen (N) and fourteen per cent (14%) of potash ( $K_2O$ ).

### *Official, First Action* GUARANTEEING IN TERMS OF ELEMENTS

All fertilizer components with the exceptions of potash ( $K_2O$ ) and phosphoric acid ( $P_2O_5$ ) if guaranteed shall be stated in terms of the elements.

### RECOMMENDATION

Owing to the fact that instances of serious injury to livestock has resulted, it



is recommended that bags of fertilizer nitrates shall carry the warning "*injurious to livestock.*"

#### *Proposed Definitions*

##### MAGNESIA (MAGNESIUM OXIDE)

(1) *Magnesia (magnesium oxide)* is a product consisting chiefly of the oxide of magnesium. Its grade shall be stipulated. For example: Magnesia—75% MgO.

##### NITRATE OF AMMONIA ( $\text{NH}_4\text{NO}_3$ )—AMMONIUM NITRATE

(2) *Nitrate of Ammonia ( $\text{NH}_4\text{NO}_3$ )—ammonium nitrate* is a product composed chiefly of nitrate of ammonia. Its nitrogen content shall be stipulated. For example: Ammonium nitrate—30% N.

##### FUSED TRICALCIUM PHOSPHATE

(3) *Fused tricalcium phosphate* is a glassy material resulting from the quenching of substantially defluorinated fusions of rock phosphate. It shall be of such fineness that ninety per cent (90%) will pass an 80-mesh screen and eighty-five per cent (85%) of its  $\text{P}_2\text{O}_5$  content shall be "available."

##### CALCIUM METAPHOSPHATE

(4) *Calcium metaphosphate* is a glassy product that is composed chiefly of the phosphate indicated by the formula  $\text{Ca}(\text{PO}_3)_2$ . It shall be of such fineness that ninety per cent (90%) will pass a 20-mesh sieve, and its content of available  $\text{P}_2\text{O}_5$  equivalence shall be stipulated. Example: Calcium metaphosphate—60 per cent available  $\text{P}_2\text{O}_5$ .

##### POTASSIUM METAPHOSPHATE

(5) *Potassium metaphosphate* is a product represented by the formula  $\text{KPO}_3$ . It shall be of such fineness that ninety per cent (90%) will pass a 50-mesh screen, and its content of  $\text{P}_2\text{O}_5$  and  $\text{K}_2\text{O}$  equivalence shall be stipulated. For example: Potassium metaphosphate—58 per cent  $\text{P}_2\text{O}_5$ ; 32 per cent  $\text{K}_2\text{O}$ .

## REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES\*

By E. L. GRIFFIN (Office of Distribution, War Food Administration, Washington, D. C.), *Chairman*; L. S. WALKER† and H. H. HANSON‡

### ENZYMES

It is recommended that this work be discontinued.

### FEEDING STUFFS

It is recommended—

(1) That the method for sampling feeding stuffs submitted by the Referee be adopted as tentative (see p. 89).

(2) That the temperature of ashing—600°C.—be adopted as official (final action).

(3) That work on the determination of calcium and iodine in mineral mixed feeds be continued.

(4) That an associate referee on the determination of mineral constituents of mixed feeds be appointed.

\* These recommendations, submitted by Subcommittee A, were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis, A.O.A.C.*, 1940.

† Served for G. E. Grattan.

‡ Served for H. A. Halvorson.

- (5) That the work on lactose in mixed feeds be continued.
- (6) That the work on fat in fish meal be continued.
- (7) That work on methods of detecting the adulteration of condensed milk products be continued.
- (8) That the method for the detection of starch in condensed or dried milk products, made official (first action) at the last meeting, now be made official (final action) and that study of the method be discontinued.
- (9) That the study of fat in cooked animal feeds containing cereals be continued.
- (10) That the work on crude fat or ether extract be continued.
- (11) That the work on filtration aids in crude fiber determination be continued.
- (12) That the method for soluble chlorine published in *This Journal*, 26, 89, be adopted as official (first action), and that the study be continued.
- (13) That the method for the determination of ammoniacal and urea nitrogen, published in *This Journal*, 24, 79; 25, 93, be made official (final action).
- (14) That the study of methods for evaluating the activity of yeast be continued.
- (15) That work on microscopic examination of feeding stuffs be inaugurated.
- (16) That an associate referee on fluorine in feeds be appointed and that in the study of methods consideration be given to utilizing present procedures for fluorine in other substances as far as practicable.

#### FERTILIZERS

It is recommended—

- (1) That the slotted single-tube sampler, the slotted double-tube sampler, and the slotted tube and rod sampler, *This Journal*, 24, 501, all with solid pointed ends, be adopted for sampling fertilizers (official, final action).
- (2) That paragraph 41(a) and (b), p. 31, of the official potash method, be changed to provide for the use of factor weight by adding "or the factor weight 2.425 g" after "2.5 g" in the first and second lines, respectively, and that to 42(a) there be added, "If the factor weight and a 50 ml aliquot (containing 0.485 g sample) are used, multiply weight by 40 to get per cent  $K_2O$ " (official, final action).
- (3) That the official method for the determination of water-insoluble organic nitrogen, 34, p. 29, be deleted (final action).
- (4) That for section (b) of the official method for the determination of nitrate nitrogen (31, p. 28) the following directions be substituted: "(b) Determine water-insoluble organic nitrogen as directed under 35 but use 2.5 g of the mixed fertilizer" (official, final action).

(5) That the heading "Total Nitrogen," preceding paragraphs 24, 25, 26, and 27 be changed to read, "Total Nitrogen in Mixed Fertilizers," (official, final action).

(6) That the words "in mixed fertilizer" be added to the heading "Nitrate Nitrogen," preceding par. 31, p. 28 (official, final action).

(7) That in line 6 of par. 60, p. 38, after the words "for unmixed nitrate salts," the words "or for mixed fertilizers containing considerable nitrate nitrogen" be added (official, final action).

(8) That the long volumetric method for the determination of copper, adopted as official (first action) in 1941 and published in *This Journal*, 24, 67, be adopted as official (final action).

(9) That the statement ("Not applicable to samples containing free ammonia or compounds other than water that are volatilized at the temperature of drying"), be added to the method for the determination of moisture by drying (4, p. 20), (official, final action).

(10) That to Chapter II, the paragraph relating to graduated flasks published in *This Journal*, 25, 78, be added, official (final action).

(11) That in the official Devarda method (30, p. 27), the quantity "0.5 g" be changed to "0.35 or 0.5 g"; that the last paragraph be changed to read, "In the analysis of nitrate salts, dissolve 3.5 or 5.0 g in  $H_2O$ , make up to 250 ml, and use 25 ml"; and that the same sentence be added to the ferrous sulfate-zinc-soda method for the same determination (29, p. 27), (official, final action).

(12) That the following sentence be added to the method for the preparation of standard sodium or potassium hydroxide solution (10(b), p. 22): "Burets in constant use are likely to become so corroded as to increase their capacity, and therefore should be tested at least once a year" (official, final action).

(13) That the following clarifying paragraph be added to the method for the determination of potash in nitrate of potash, as 41(e), p. 31: "Nitrate of potash or nitrate of potash and soda.—If impure, proceed as directed in (a); if sufficiently pure, proceed as directed for potash salts (b), except to evaporate an aliquot to dryness in a porcelain dish with 2 ml. of HCl (if Pt dish is used, add  $H_2SO_4$  instead) and to take up with  $H_2O$  and a few drops of HCl before adding the Pt solution" (official, final action).

(14) That Method III, volumetric modification of the method for the determination of acid-soluble magnesium, 54, p. 36, adopted as official (first action), (*This Journal*, 24, 47) be adopted as official (final action).

(15) That the bismuthate method for the determination of acid-soluble manganese (*This Journal*, 24, 69, adopted as official (first action), *ibid.*, 25, 49, be adopted as official (final action).

(16) That the official (first action) volumetric periodate method for the determination of manganese (56 and 57, p. 37) be deleted (final action).

(17) That the colorimetric method for the determination of manganese (58, p. 37), adopted as official (first action), (*This Journal*, 24, 47) and corrected editorially (*ibid.*, 25, 49 and 79), be adopted as official (final action).

(18) That Method I, official, for the determination of acid-soluble magnesium (52, p. 35) be deleted, but that such portions as are referred to in Method II (53, p. 36) be incorporated in Method II when revised (official, first action).

(19) That titration with a glass electrode be adopted as tentative in the method for the determination of acid- and base-forming quality, as recommended by the associate referee (see p. 73).

(20) That methods for the determination of phosphoric acid in basic slag be included with the other methods for phosphoric acid in fertilizers (*Methods of Analysis*, A.O.A.C., 1940). For the changes necessary to make this presentation possible see p. 73 (20).

(21) That the study of the determination of moisture be continued.

(22) That the study of the official ammonium citrate method for determining available  $P_2O_5$  in fertilizers (15, p. 23) be continued.

(23) That work on the ferric sulfate and dipotassium phosphate method for the determination of nitrogen be discontinued and that the use of sodium phosphate of suitable pH be studied to ascertain whether it is better than magnesium oxide for ammoniacal nitrogen (28, p. 27).

(24) That methods for the determination of acid-soluble magnesium, magnesium activity, and acid-soluble manganese be further studied.

(25) That work be continued on the method for determining acid- and base-forming qualities of fertilizer recommended by the associate referee, including the method for eliminating basicity due to coarse material and the temperature of ignition.

(26) That work on the details of the method for the determination of potash be continued, including the method for recovery of platinum.

(27) That search for new methods for the determination of sulfur be continued and that the method for the determination of calcium (47, p. 34; *This Journal*, 24, 67) be further studied.

(28) That collaborative work be done on the volumetric method for the determination of total zinc in samples containing less than 0.1 per cent, published in *This Journal*, 26, 67, with the objective of substituting it for the colorimetric method.

The Subcommittee does not approve the referee's recommendation that the method for the determination of citrate-insoluble phosphoric acid in non-acidulated samples other than basic slag (16(b), p. 24) be changed to read as follows: "*Dicalcium phosphate*.—Place 1 g of the sample on a 9 cm qualitative filter paper in a 250 ml Erlenmeyer flask and proceed as directed in 16(a)," but recommends that it be referred back to the referee for further consideration.

## INSECTICIDES AND FUNGICIDES

It is recommended—

(1) That the value, 1 ml  $M/100$   $KIO_3$  = 5.70 mg of Pyrethrin I, be adopted as the factor in the Mercury Reduction Method for Pyrethrin I in pyrethrum powder and extracts (official, first action).

(2) That procedures 1, 2, 3, 4, and 5, as proposed by the associate referee (see p. 74), be adopted as a substitute for the lead chlorofluoride method for total fluorine (official, first action).

(3) That the study of naphthalene in poultry lice products be discontinued.

(4) That study on rodenticides be inaugurated.

(5) That methods for the determination of nicotine be studied.

(6) That an associate referee be appointed to study D.D.T.

## DISINFECTANTS

It is recommended that this work be continued.

## LEATHERS AND TANNING MATERIALS

It is recommended that this work be continued.

## VITAMINS

It is recommended—

(1) That the thiochrome method for the determination of vitamin  $B_1$  proposed by the associate referee be adopted as a tentative method (see p. 103).

(2) That the method for the determination of vitamin C proposed by the associate referee be adopted as a tentative method (see p. 102).

(3) That fluorometric methods for the determination of riboflavin be further studied so that such a method may be recommended for materials other than yeast, dried skim milk, and alfalfa.

(4) That the microbiological method for riboflavin be further studied to improve the basal medium and also with a view to making the procedure identical with the procedure that has been adopted by the U. S. Pharmacopoeia.

(5) That the method for the determination of nicotinic acid proposed by the associate referee be adopted as tentative (see p. 105).

(6) That the method for the determination of crude carotene (61, p. 369) be extended to materials other than dried hays and dried plants as provided by the modifications described by the associate referee (see p. 108).

(7) That the complete chromatographic method for the determination of carotene described by the associate referee be adopted as tentative (see p. 107).

(8) That studies be continued on chemical and physical methods for vitamin A.

(9) That studies be continued on vitamin D in milk and for poultry.

(10) That studies be continued on methods for vitamin K.

(11) That studies be inaugurated on methods for pantothenic acid.

## STANDARD SOLUTIONS

It is recommended—

(1) That the methods of standardization of thiocyanate solution be further studied.

(2) That the method for preparation and standardization of sodium thiosulfate solution be further studied.

(3) That the method for the preparation of standard hydrochloric acid solution from constant boiling acid, *This Journal*, 25, 110) be adopted as official (final action).

(4) That the method for standardizing sodium hydroxide solutions by means of constant boiling hydrochloric acid (*Ibid.*, 112) be adopted as official (final action).

(5) That the method for the standardization of potassium permanganate solution be further studied with particular reference to the criticism suggested by the Referee on Synthetic Drugs.

(6) That methods for the determination of standard buffer solutions be studied.

## PLANTS

It is recommended—

(1) That the studies on sampling be continued.

(2) That the studies on iodine and boron be continued.

(3) That the studies on carbohydrates be continued.

(4) That the studies on copper and cobalt be continued and collaborative work be initiated as soon as conditions permit.

(5) That the work on carotene and chlorophyll be continued.

(6) That the study of the *o*-phenanthroline procedure for the colorimetric and the titanous chloride method for the titrametric evaluation of iron be continued, with the view to adopting them as official methods in place of the thiocyanate method as soon as necessary conditions have been met.

(7) That further study be given to the ashing of plant material and extraction of the ash for the analysis of iron.

(8) That the calibration of a photoelectric colorimeter by means of a plant extract, as described in the paper by Benne, Rose, and Colmar (to be published later in *This Journal*), be given further study and applied to different makes of instruments.

(9) That the two methods for chlorophyll recommended by the associate referee be adopted as tentative (see p. 77).

## SOILS AND LIMING MATERIALS

It is recommended—

(1) That the method for the titrative evaluation of slags submitted by the associate referee be adopted as tentative and studied further.

(2) That the technic previously proposed for the distillation method of determining fluorine be studied collaboratively.

(3) That the "two-point" titration procedure for the determination of exchangeable hydrogen in soils be studied further in relation to soil calcium carbonate reactions.

(4) That the work on the extraction of exchangeable calcium and magnesium in the presence of carbonates be continued.

(5) That the work on boron be continued as previously outlined by the referee.

(6) That further study be made of methods for the determination of copper in soils.

(7) That studies of methods for the determination of zinc in soils be continued.

(8) That work on hydrogen-ion concentration in arid and semi-arid soils be continued.

#### PAINTS

It is recommended that study on paints be discontinued for the present.

### REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES\*

H. J. FISHER (Agricultural Experiment Station, New Haven, Conn.),  
*Acting Chairman*; W. F. REINDOLLAR and DAN DAHLE†

#### NAVAL STORES

It is recommended that the subject be continued.

#### RADIOACTIVITY

It is recommended that the subject be continued.

#### VEGETABLE DRUGS AND THEIR DERIVATIVES

The referee reported that owing to wartime conditions, little work could be done by the associate referees. Of the thirteen projects assigned, he recommends that two be discontinued, one reassigned, and the remainder continued. The Committee approves the recommendations.

#### CHEMICAL METHODS FOR ERGOT ALKALOIDS

The associate referee reported that a chemical method for the assay of ergometrine had been devised and compared with the results of bioassays. He recommended that the method be studied collaboratively. The Committee concurs in this recommendation.

#### THEOPHYLLINE SODIUM SALICYLATE

It is recommended that the topic be reassigned.

#### PHYSOSTIGMINE IN OINTMENTS

The associate referee reported that work had been completed to the point where a collaborative study could be made. He recommends that the subject be continued. The Committee concurs.

\* These recommendations, submitted by Subcommittee B, were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis*, A.O.A.C., 1940.

† Acting for A. E. Paul.

## QUININE ETHYL CARBONATE

No report was received, though it is understood that some work has been done. It is recommended that the subject be continued.

## THEOBROMINE AND PHENOBARBITAL

The associate referee has been trying to eliminate the interference of starch in the assay for these substances. It is recommended that the topic be continued.

## GLUCOSIDES AND SAPONINS

The associate referee found most of the methods in the literature to be of the bioassay type, which he did not feel qualified to study. The referee recommends that the subject be dropped. The Committee concurs.

## PROSTIGMINE

Owing to a misunderstanding, the associate referee worked on Method I, which is a bromide determination and not specific for prostigmine. He submitted a report showing excellent results by this method. Because the associate referee, the referee, and the Committee believe that Method II, which depends on the determination of dimethylamine obtained by hydrolysis of prostigmine, is a more specific method, the Committee now recommends that Method II be studied further.

## STRYCHNINE IN PILLS

It is recommended that the topic be discontinued.

## QUININE AND STRYCHNINE

The referee recommends that the subject be continued. The Committee concurs.

## POLAROGRAPHIC METHODS

The referee recommends that the subject be continued. The Committee concurs.

## AMINOPYRINE, EPHEDRINE, AND PHENOBARBITAL

It is recommended that study of these drugs be continued.

## SYNTHETIC DRUGS

The referee reports that of the fourteen topics assigned to him work on two has been completed and methods are recommended for adoption; for three, methods of assay have been adopted by the U.S.P. or the N.F. and it is recommended that these topics be dropped. In the case of the other ten topics, further study is recommended. Six new topics are recommended for study: Demerol, propadrine, carbromal, phenolsulfonphthalein, procaine, and sulfobromophthalein. The referee also recommends changes in the status of a number of methods now tentative or official (first action). These methods are listed later in this report. The Committee concurs in the recommendations of the referee.



## PLASMOCHINE

No report was received, although the associate referee has submitted samples for collaborative study. It is recommended that the subject be continued.

## PHENOTHIAZINE

It is recommended that the subject be continued.

## BENZEDRINE

No report was received, and it is understood that no further work has been done. The referee recommends that the study of the determination of benzedrine in inhalants be continued. The Committee concurs.

## HYDROXYQUINOLINE SULFATE

It is recommended that the topic be reassigned.

## METHYLENE BLUE

A report of the associate referee was published in *This Journal*, 26, 242. He finds that the combination of the A.O.A.C. method of separation with the perchlorate method of determination yields satisfactory results with certain mixtures but not with others. He recommends that the topic be continued. The referee and the Committee concur.

## ETHYL AMINOBENZOATE

The referee recommends that the topic be reassigned. The Committee concurs.

## METRAZOL

No report was received, and it is understood that no further work has been done. It is recommended that the topic be reassigned.

## BROMOBARBITURATES AND THIOBARBITURATES

Two methods were studied. Both gave satisfactory results. One was the present official method for barbital and phenobarbital; the other method employs chloroform alone as a solvent. The associate referee recommends the tentative adoption of both methods for most of the barbiturates. The Committee recommends that the present official method (*Methods of Analysis*, A.O.A.C., 1940, p. 574, 44) be amended (first action) as follows:

- (1) That the title of the method be changed to "Barbiturates."
- (2) That in 44(b) the statement "chloroform alone may be used except with nostal and pernoston" be added in parentheses.

## ACETANILID

No work was done. The referee recommends that the topic be reassigned. The Committee concurs.

## SULFANILAMIDE DERIVATIVES

No work has been done on this topic for the last two years. It is recommended that the subject be reassigned.

## PHENOLPHTHALEIN AND BILE SALTS

The associate referee has done considerable work on this subject, but he is not yet ready to submit a method to collaborative study. It is recommended that the topic be continued.

## ATABRINE

The associate referee worked out a method for the determination of this compound which gave good results on collaborative study. As the First Supplement to the Pharmacopoeia is about to adopt a similar method, the Committee recommends that further study of this method be discontinued but that the short method suggested by the referee be studied.

## SEDORMID

The associate referee devised a method for determination of the compound which depends upon its hydrolysis to urea with further hydrolysis of the urea to ammonia. Direct determination by solvent extraction was also found to give good results, but the associate referee did not believe this method was sufficiently specific. He has not yet submitted the urea method to collaborative study, and recommends that the topic be continued. The Committee concurs in the recommendation that the topic be continued, but questions the necessity of so elaborate a method as the urea method since the identity of the drug can be established by means of a melting point after it has been isolated.

## DIETHYL STILBESTROL

Methods of assay are to appear in the Supplement to the U.S.P. XII. It is recommended that the subject be dropped.

## CINCHOPHEN AND NEOCINCHOPHEN

The National Formulary and the U. S. Pharmacopoeia contain methods for the assay of cinchophen and neocinchophen tablets, respectively. For this reason it is recommended that the subject be discontinued.

## SODIUM DIPHENYLHYDANTOIN

This subject was assigned two years ago. The associate referee did no work because the U.S.P. adopted a method of assay. It is recommended that the topic be discontinued.

## MISCELLANEOUS DRUGS

The referee reports that four associate referees submitted reports for 1942, which have been published (*This Journal*, 26, 96, 99, 238, 311). For the current year two associate referees have reported. In the case of four reports recommendations for the adoption of methods were made. The Committee approves the report of the referee.

## MICROCHEMICAL TESTS FOR ALKALOIDS AND SYNTHETICS

The associate referee recommends the adoption as tentative of tests for choline and sulfadiazine already published (*This Journal*, 26, 96). The referee and Committee concur.

The associate referee has also worked out tests for quinacrine, totaquine and sodium diphenylhydantoin, but they have not yet been submitted to collaborative study. He recommends that these compounds be studied collaboratively this coming year. The Committee concurs in the recommendation.

#### EMULSIONS

Two methods for the determination of phenolphthalein in emulsions were studied collaboratively. The referee recommends that the iodination method (*This Journal*, 26, 312) be adopted as tentative. The Committee concurs.

#### SPECTROPHOTOMETRIC METHODS

The associate referee worked out methods for the determination of quinine and quinacrine, the first of which was published in *This Journal*, 26, 238. Recommendation is made that both these methods be adopted as tentative. The Committee concurs.

#### GLYCOLS AND RELATED COMPOUNDS

The report of the associate referee was published in *This Journal*, 26, 99. The tests outlined in the report were not studied collaboratively. The referee recommends that a new associate referee be appointed since the present associate referee is no longer in government service. The Committee concurs.

It is recommended that study be continued on the following subjects: Mercury Compounds (Ethanamine Method); Separation of Bromides, Chlorides, and Iodides; Thyroid; Compound Ointment of Benzoic Acid; Alkali Metals; and Preservatives and Bacteriostatic Agents in Ampul Solutions.

#### NEW TOPICS

It is recommended that the following new topics be studied:

Demerol  
Propadrine Hydrochloride  
Carbromal  
Phenolsulfonphthalein  
Procaine  
Sulfobromophthalein

#### DRUG BIOASSAYS

The referee left the government service, and no report was received. It is recommended that the subject be reassigned, and that except for glucosides and saponins present studies be continued.

#### SUMMARY OF CHANGES IN STATUS OF METHODS

It is recommended that the following methods be advanced from official (first action) to official (final action):

Monobromated Camphor, Method II (51, p. 576).  
Pilocarpine Hydrochloride (95, p. 589).  
Calomel in Calomel Ointment (192, p. 620).  
Hypophosphites in Sirup (180, p. 617).  
Salicylic Acid in Presence of Other Phenols (38, p. 572).  
Tetrachlorethylene in Mixtures (135, p. 604).

It is recommended that the following methods be advanced from tentative to official (first action):

Acetophenetidin and Caffeine (16, p. 565).  
Acetylsalicylic Acid, Acetophenetidin and Caffeine (32, p. 570).  
Bismuth Compounds in Tablets (178, p. 617).  
Calcium Gluconate (179, p. 617).  
Effervescent Potassium Bromide with Caffeine (202, p. 623).  
Iodine (183, p. 618).  
Mandelic Acid (154, p. 610).  
Oil of Chenopodium (208, p. 625).  
Phenolphthalein in Chocolate Preparations (162, p. 613).  
Sulfanilamide (168, p. 614).  
Theophylline (107, p. 593).

#### COSMETICS AND COAL TAR COLORS

The referee reports that several of the associate referees are no longer on cosmetic work, some of them being in the armed forces. Nevertheless, five reports on cosmetics and four on coal tar colors have been presented, and all but two of these reports have been published in *This Journal*.

The referee recommends changes in the status of certain methods now tentative, or official (first action) and the adoption as tentative of two other methods. He also recommends dropping five of the present topics and assigning three new topics. These recommendations are discussed later in this report.

The Committee approves the report of the referee.

#### MOISTURE IN COSMETICS

The associate referee recommends continuation of the topic. The referee and the Committee concur.

#### COMMON ASH CONSTITUENTS

The associate referee is out of this type of work. The referee recommends that the topic be discontinued. The Committee concurs.

#### ARSENIC IN HAIR LOTIONS

The associate referee studied the application of various methods for the determination of arsenic in hair lotions. He finds the official Gutzeit method and all modifications of it studied to be unsuitable, but reports satisfactory results with the Cassil-Wichmann microprocedure (*This Journal*, 22, 436). He recommends further study. The referee and the Committee concur.

LEAD IN COSMETICS

The referee recommends that the topic be continued. The Committee concurs.

MERCURY SALTS IN COSMETICS

The associate referee is no longer doing this type of work. The referee recommends reassignment of the topic. The Committee concurs.

ALKALIES IN CUTICLE REMOVERS

The associate referee recommends continuation of the topic. The referee and the Committee concur.

PEROXIDES IN COSMETICS

The associate referee is now doing other work. The referee recommends that this topic be discontinued. The Committee concurs.

BETANAPHTHOL IN HAIR LOTIONS

The associate referee is no longer doing this type of work. The referee recommends discontinuation of the topic for the duration. The Committee concurs.

PYROGALLOL IN HAIR DYES

The associate referee recommends continued study. The referee and the Committee concur.

RESORCINOL IN HAIR LOTIONS

The associate referee recommends that the study be continued. The referee and the Committee concur.

SALICYLIC ACID IN HAIR LOTIONS

The associate referee, at the last meeting, submitted a method as tentative and it was so adopted (*This Journal*, 25, 112). In his present report he has revised this tentative method in accordance with suggestions made by collaborators and submitted the revised method for collaborative work. The revised method was published in *This Journal*, 26, 355. The associate referee recommends that the present method be adopted as official (first action). The referee and the Committee concur in these recommendations.

COSMETIC CREAMS

The associate referee recommends continued study. The referee and the Committee concur.

COSMETIC POWDERS

The referee recommends continued study. The Committee concurs.

DENTIFRICES AND MOUTHWASHES

The associate referee recommends discontinuance or the assignment of a more specialized topic. In view of the present wartime use of substitute

materials in these products, the referee recommends discontinuance for the duration. The Committee concurs.

#### UREA IN DEODORANTS

The associate referee recommends continued study. The referee and the Committee concur.

#### DEODORANTS AND ANTI-PERSPIRANTS

This topic has no assigned associate referee. The referee recommends continuance and assignment to an associate referee as soon as one can be found. The Committee concurs.

#### DEPILATORIES

At the last meeting of the Association the associate referee recommended that a method for the determination of sulfides in depilatories be adopted as official (first action). The method was so adopted (*This Journal*, 25, 113). This method is now recommended for adoption as official (final action) on the basis that it has been in use since 1941 with no reports of unsatisfactory results. The referee and the Committee concur.

#### HAIR DYES AND RINSES

Two methods for the determination of *p*-phenylenediamine in hair dyes were adopted as official (first action) at the last meeting (*This Journal*, 25, 113). The associate referee recommends that these be adopted as official (final action). The referee concurs.

The associate referee submitted to collaborators two methods for the determination of 2,5-diaminotoluene (*This Journal*, 26, 117). The results indicated that Method I gave satisfactory results whereas Method II did not. Since, however, Method I is not specific for 2,5-diaminotoluene, whereas Method II would be, the associate referee recommends further study. The referee concurs, but because the present associate referee is no longer employed in cosmetic work, adds the recommendation that the topic be reassigned. The Committee concurs in both recommendations.

#### HAIR STRAIGHTENERS

The associate referee recommends continued study. The referee and the Committee concur.

#### LIP MAKE-UP AND ROUGES

The associate referee is in the U. S. Army. The referee recommends that the topic be suspended for the duration of the war. The Committee concurs.

#### EYE LOTIONS

The associate referee is in the U. S. Army. The referee recommends that the topic be suspended for the duration of the war. The Committee concurs.

## MASCARA, EYEBROW PENCILS AND EYESHADOW

The associate referee reports on general methods and recommends that the topic be continued with a study of more specialized sub-topics. The referee and the Committee concur. The report was published in *This Journal*, 26, 317.

## NAIL COSMETICS

The associate referee has reported on preliminary separation of non-volatile constituents of nail cosmetics. Since he is no longer doing cosmetic work, he recommends reassignment of the topic. The referee and the Committee concur.

## COAL-TAR COLORS

## ETHER EXTRACT IN COAL-TAR COLORS

The associate referee recommends continued study. The referee and the Committee concur.

## PURE DYE, IMPURITIES, AND SUBSTRATA IN PIGMENTS

The associate referee recommends continued study. The referee and the Committee concur.

## BUFFERS AND SOLVENTS IN THE TITANIUM TRICHLORIDE TITRATION

The associate referee reported at the last meeting on three methods (*This Journal*, 25, 114). Two of these (for D&C Orange No. 3 and D&C Yellow No. 7) were adopted as official (first action). These are now recommended for adoption as official (final action). The referee and the Committee concur. The third method (for D&C Green No. 5) was adopted as tentative. The associate referee recommends that this method be retained as tentative for another year to permit further study. The referee and the Committee concur.

In his present report the associate referee has studied collaboratively methods for (1) pure dye in D&C Red Nos. 8 and 31 and (2) pure dye in D&C Red No. 19. He recommends that the first method be adopted as tentative and that the second be given further study. The referee and the Committee concur.

## HALOGENS IN HALOGENATED FLUORESCINS

The associate referee submitted to collaborators three samples of halogenated fluorescein dyes for analysis by the method presented in 1941 (*This Journal*, 25, 757). The results indicated that proper technic would produce accurate and reproducible results. The associate referee proposes, however, to rewrite the method, laying greater stress on such directions as are important to obtain correct results (see p. 112). He recommends the adoption of the method as tentative and the referee concurs.

The Committee approves the adoption of the method as tentative after clarification of the wording.

## INTERMEDIATES IN CERTIFIED COAL-TAR COLORS

The associate referee is now in the U. S. Navy. Since the study of the topic is of great importance, the referee recommends its reassignment. The Committee concurs.

## SPECTROPHOTOMETRIC TESTING OF COAL-TAR COLORS

The associate referee recommends continued study. The referee and the Committee concur.

## SUBSIDIARY DYES IN D&amp;C COLORS

The associate referee reports on a method for the determination of 1,4-(*o*-sulfo-*p*-toluino)-anthraquinone in D&C Green No. 5. He recommends that the topic be continued and the presented method studied collaboratively. The referee and the Committee concur.

## IDENTIFICATION OF CERTIFIED COAL-TAR COLORS

The referee recommends continued study. The Committee concurs.

## ALIZARIN IN MADDER LAKE

The associate referee has studied collaboratively the method he presented at the last meeting. The results indicate that the method is satisfactory. He therefore recommends its adoption as official (first action). The referee and the Committee concur. The report was published in *This Journal*, 25, 956; 26, 242.

SUMMARY OF CHANGES IN STATUS OF METHODS FOR COSMETICS  
AND COAL-TAR COLORS

It is recommended that the following methods be advanced from the status of official (first action) to official (final action):

Sulfides in depilatory powders (*This Journal*, 25, 113)

Paraphenylenediamine in hair dyes and rinses (*Ibid.*)

Pure dye in D&C Orange No. 3 (*Ibid.*, 114)

Pure dye in D&C Yellow No. 7 (*Ibid.*)

## NEW TOPICS

It is recommended that the following new topics be studied:

Cosmetic skin lotions

Mixtures of coal-tar colors for drugs and cosmetics

Acetates, carbonates, halides, and sulfates in certified colors

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REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS  
OF REFEREES\*

W. B. WHITE (U. S. Food and Drug Administration, Washington, D. C.),  
Chairman; J. O. CLARKE, and C. S. LADD

METALS IN FOODS

It is recommended—

- (1) That studies on micro methods for the determination of copper be continued.
- (2) That the general method for the determination of fluorine submitted by the associate referee be adopted as tentative (see p. 90).
- (3) That the rapid method for the determination of fluorine as spray residue on apples and pears be adopted as tentative (see p. 98).
- (4) That studies be continued on methods for the determination of lead in special products.
- (5) That studies be initiated on the preparation of samples of large size for the rapid determination of spray residues on fruits and vegetables.
- (6) That the tentative method for the determination of selenium (*This Journal*, 26, 346) be adopted as official (first action).
- (7) That studies on methods for the determination of zinc be continued.
- (8) That methods for the determination of mercury and cadmium be studied.
- (9) See also Recommendation (14) under Alcoholic Beverages.

EGGS AND EGG PRODUCTS

It is recommended—

- (1) That the official (first action) method for the determination of cholesterol (*This Journal*, 25, 87) be adopted as official (final action).
- (2) That studies on chemical methods for the detection and measurement of decomposition in eggs and egg products be assigned to a referee on decomposition in foods.
- (3) That the open Carius method for the determination of salt in egg products (*This Journal*, 26, 352) be adopted as an alternative official method (first action).

MEAT AND MEAT PRODUCTS

It is recommended—

- (1) That studies of methods for the detection and determination of dried skim milk and soybean flour be continued.
- (2) That the tentative method for the detection of agar agar (*This Journal*, 25, 93), be made official (first action).

COLORING MATTERS IN FOODS

It is recommended—

- (1) That the method for the detection of minute amounts of FD&C

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\* These recommendations, submitted by Subcommittee C, were approved by the Association, and unless given otherwise all references are to *Methods of Analysis*, A.O.A.C., 1940.

Yellow No. 5 (tartrazine) presented by the referee be studied collaboratively.

(2) That investigational work be continued on the quantitative separation and estimation of FD&C Yellow No. 5 (tartrazine) and FD&C Yellow No. 6 (sunset yellow FCF).

(3) That investigational work be undertaken to separate and determine quantitatively mixtures of FD&C Green No. 2 (light green SF yellowish), FD&C Green No. 3 (fast green FCF), and FD&C Blue No. 1 (brilliant blue FCF).

(4) That studies be undertaken on the separation and quantitative determination in mixtures of FD&C Yellow No. 3 (Yellow AB), FD&C Yellow No. 4 (Yellow OB), FD&C Orange No. 2 (Orange SS), and FD&C Red No. 32 (Oil Red XO).

(5) That collaborative work on analytical methods for the coal-tar colors certifiable for use in foods be conducted.

#### GUMS IN FOODS

It is recommended—

(1) That in the studies on the detection of gums in food attention be also directed to the substances, both natural and synthetic, being used as substitutes for gums because of temporary scarcity.

(2) That the tentative method for the detection of gums in soft curd cheese (127-130, p. 305), be subjected to collaborative study.

(3) That studies of methods for the detection of gums in frozen desserts be continued.

(4) That the tentative method for the detection of gums in mayonnaise and French dressing (55, p. 477) be studied collaboratively.

#### CANNED FOODS

It is recommended—

(1) That studies on methods for the determination of quality factors be continued.

(2) That studies on methods of preparation of sample and determination of moisture in dried vegetables be continued.

#### OILS, FATS AND WAXES

It is recommended—

(1) That studies be made on the application to other oil seeds of the official method for refractometric determination of oil in flaxseed (63-65, pp. 448-452).

(2) That methods for the determination of unsaponifiable matter be studied with a view to adopting a method of wide applicability.

(3) That the method for the detection of olive oil in mixtures with other oils (*This Journal*, 26, 499) be studied collaboratively.

(4) That studies of methods for the determination of peanut oil be continued.

## FOOD PRESERVATIVES AND SWEETENERS

It is recommended—

- (1) That studies be conducted on methods for the determination of benzoate of soda in foods.
- (2) That methods for the determination of the esters of benzoic acids be studied.
- (3) That studies be continued on methods for the detection and determination of saccharin.
- (4) That study of methods for the determination of added sulfur dioxide compounds in comminuted meats be continued.
- (5) That studies of methods for monochloroacetic acid be continued.

## SPICES AND CONDIMENTS

It is recommended—

- (1) That the official method (first action) for the determination of total phosphoric acid in vinegar (*This Journal*, 24, 83) be made official (final action).
- (2) That the modified Lichthardt method for the detection of caramel in vinegar (*This Journal*, 26, 234) be adopted as tentative.
- (3) That the tentative method for the determination of color removed by fullers' earth (76, p. 481) be dropped.
- (4) That the method for the differentiation between vinegar and commercial acetic acid presented by the associate referee be adopted as tentative and studied collaboratively (see p. 101).
- (5) That studies be continued on methods for the determination of starch in salad dressing.
- (6) That the tentative method for the determination of starch in prepared mustard (*This Journal*, 25, 97), be further studied in regard to its applicability to mustard flour.
- (7) That the official method for the determination of salt in prepared mustard (36, p. 474) be dropped (final action).
- (8) That the official method (first action) for the determination of salt in prepared mustard be made official (final action). Except that 3–4 grams are specified as a sample, this method is the same as the modification of the open Carius method published in *This Journal*, 24, 703.

## FISH AND OTHER MARINE PRODUCTS

It is recommended—

- (1) That the several methods for the determination of total solids and ether extract (*This Journal*, 26, 226–232) be studied collaboratively with a view to selecting the most suitable method for each constituent.
- (2) That studies be continued on the methods for the determination of volatile acid published in *This Journal*, 25, 176, and be assigned under the subject of decomposition in foods.

## MICROCHEMICAL METHODS

It is recommended that study of microchemical methods be continued.

## COFFEE AND TEA

It is recommended that study be continued on methods for determining chlorogenic acid in coffee.

## MICROBIOLOGICAL METHODS

It is recommended—

(1) That the official method (first action) for the detection and estimation of numbers of thermophilic bacteria in sugars (12-18, pp. 642-643) be studied collaboratively.

(2) That the official methods (first action) for the examination of frozen egg products (1-11, pp. 639-641), as revised and published in *This Journal*, 24, 93-95, be studied collaboratively.

(3) That the official methods (first action) for the examination of canned vegetables, other than tomatoes (19-21, pp. 643-646), be studied collaboratively.

(4) That the tentative methods for the examination of nuts and nut products (*This Journal*, 25, 109) be studied collaboratively.

(5) That studies be continued on methods for the examination of canned fishery products, canned meats, canned fruit, fruit products, and tomatoes.

## DAIRY PRODUCTS

It is recommended—

(1) That studies be continued on methods for the detection of neutralizers in dairy products.

(2) That the tentative method for preparation of sample for analysis of frozen desserts containing insoluble particles (*This Journal*, 25, 85) be adopted as official (first action), and that the word "particles" be changed to "ingredients."

(3) That studies on the analysis of frozen desserts (including the quantitative determination of gelatin and other "stabilizers") be continued, and that methods for determining the apparent volume of frozen desserts be included.

(4) That studies on methods for sampling cheese and for preparation of sample be continued.

(5) That studies on methods for the determination of mold mycelia in butter (108-9, p. 300), be continued.

(6) That studies on methods for detecting decomposition in dairy products be assigned to a general referee on decomposition in foods.

(7) That the tentative method for the determination of lactic acid (*This Journal*, 20, 134; 25, 255; 26, 199) be further studied.

(8) That the double dilution method for correcting the volume of precipitate in the optical determination of lactose in milk (16, p. 271; *This Journal*, 25, 608) be changed to provide for the use of a weighed sample of 65.8 (2 N weight) in place of a calculated measured sample, and adopted as official (first action).

- (9) That studies on ash in milk and evaporated milk be continued.
- (10) That studies on pasteurization of milk and cream be continued.
- (11) That methods for the determination of fat in cheese be studied.

#### EXTRANEOUS MATERIAL IN FOOD

It is recommended that methods for the separation and identification of extraneous material in food be studied and referees appointed.

#### FILL OF CONTAINER

It is recommended that studies be continued on methods of fill of container of foods, drugs, and cosmetics.

### REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES\*

W. C. JONES (Department of Agriculture, Richmond, Va.), *Acting Chairman*; R. A. OSBORN†, and JOSEPH CALLAWAY‡

#### ALCOHOLIC BEVERAGES

MALT, HOPS, CEREAL ADJUNCTS, BREWING SUGARS AND SIRUPS, AND BEER

It is recommended—

- (1) That the method for the determination of bushel weight described in the report of the associate referee for 1943 be adopted as an alternative tentative method (see p. 81). (This method is not to be used to determine bushel weight for the purpose of determining compliance with requirement of U. S. Grain Standards Act.)
- (2) That the methods for the analysis of caramel malt and black malt published in *This Journal*, 26, 300–301, be adopted as tentative.
- (3) That the ferricyanide modification for the determination of reducing sugars after diastasis be adopted as a tentative optional procedure in the method for determining diastatic power (48, pp. 160–161) and that this procedure be inserted in the method with the changes in wording suggested by the associate referee (see p. 82).
- (4) That the methods for the sampling of hops and for the determination of leaves, stems, seeds, moisture, and total soft (alpha and beta) and hard (gamma) resins in hops, published in *This Journal*, 25, 292–296, and revised as shown in the associate referee's report for 1943 be adopted as tentative (see p. 83).
- (5) That the study of analysis of cereal adjuncts be continued.
- (6) That the methods for the determination of moisture, extract, fermentable extract, and protein, published with the methods of analysis of the American Society of Brewing Chemists (1940), pages 2–8, under "Brewing Sugars and Sirups," be adopted as tentative and that the method for fermentable extracts be subjected to collaborative study.

\* These recommendations, submitted by Subcommittee D, were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis*, A.O.A.C., 1940.

† Acting for J. W. Sale.

‡ Acting for J. A. LeClerc.

(7) That the modification of the tentative method for the determination of dextrin in beer (13, p. 152) recommended by the associate referee be further studied.

(8) That the tentative method for the determination of chlorides in beer (26, p. 155) be made official (final action).

(9) That the methods for pH and acidity in beer described in the report of the referee for 1943 be further studied.

(10) That the method for the determination of sulfur dioxide in beer (21, p. 154) be made official (final action).

(11) That the tentative method for the determination of iron in beer (*This Journal*, 24, 216) and also other methods for this determination be further studied.

(12) That methods applicable to the determination of copper in beer be further studied, and that consideration be given to the dithizone carbonate method outlined in the report on copper and selenium in beer for 1943.

(13) That methods applicable to the determination of tin in beer be studied.

(14) That the associate referees on inorganic elements in beer confer with the referees on metals in foods and on iron in cereals with a view to the unification of directions for the determination of iron, copper, tin, and selenium.

(15) That the method for the determination of color in beer (2, p. 150) be made official (final action).

(16) That the method for determining turbidity in beer described in the report of the associate referee for 1943 be further studied.

(17) That photoelectric methods for the determination of color and turbidity in beer be studied.

(18) That the tentative method for the determination of carbon dioxide in beer (19, p. 152) be further studied.

(19) That study be made of methods for the determination of alpha- and beta-amylase.

#### DISTILLED LIQUORS

It is recommended—

(1) That the following methods be studied:

(a) Use of ultraviolet light in the detection of foreign material added to distilled liquors.

(b) Test for caramel referred to in the report on whiskey and rum for 1943.

(c) Chemical procedures for alcohol in brandy and other distilled spirits.

(d) Methods for detecting small quantities of impurities in alcohol.

(2) That the buffer solutions used for electrometric pH determination be collaboratively investigated and standardized.

(3) That collaborative work on the effect of proof on pH determination be investigated.

(4) That further collaborative work be done on samples such as gin and alcohol with the view to refining the technic.

(5) That study on methods for the analysis of cordials and liqueurs be continued.

#### WINES

It is recommended—

(1) That the following methods be studied:

(a) Spectrophotometric examination of wines.

(b) Detection of caramel color in wines of all kinds.

(c) The use of ultraviolet light in the detection of added foreign materials in fruit wines (*This Journal*, 25, p. 220).

(2) That the tentative method for the determination of sulfurous acid in wines (38, p. 170) be modified as recommended by the associate referee (*This Journal*, 25, 82) and made official (special action for final adoption).

(3) That the study of formol titrations in wines be continued.

#### CACAO PRODUCTS

It is recommended—

(1) That work on the determination of lecithin be continued.

(2) That work on the determination of pectic acid in cacao products be continued.

(3) That the proposed modified method for the determination of milk proteins in milk chocolate (*This Journal*, 25, 83) be made official (final action).

(4) That the tentative method for the determination of milk protein (9, p. 201) be deleted.

(5) That the study of methods for the determination of chocolate constituents of cacao products be continued and that special attention be given to products containing starch, cereal, or soybean solids.

(6) That a study be made of, and collaborative work be done on, the unified method for the determination of fat (*This Journal*, 18, 455) and that it be applied to the determination of fat in cacao products in comparison with the modified Roesse-Gottlieb method (91(b), p. 292, further modified to use a larger sample), for the purpose of improving the method for the determination of fat in products where ordinary extraction methods are difficult.

(7) That the method for calculating milk fat in milk chocolate (14, p. 204) be deleted.

#### CEREAL FOODS

It is recommended—

(1) That the method for the determination of iron on which collaborative work was reported by the referee be adopted as tentative (see p. 86), and that study be continued and conference held with other referees studying methods for iron.

(2) That the method for the determination of calcium in cereals on which collaborative work was reported by the referee be adopted as tentative (see p. 87), and that study be continued.

(3) That the study of methods for the detection and determination of rye flour in rye bread and mixtures of cereal flour be continued.

(4) That the electrometric procedure for the determination of hydrogen-ion concentration in cereal products (*This Journal*, 26, 109) be adopted as tentative, and that study be continued.

(5) That the Hopkins revision of the Mannich-Lenz procedure for the determination of starch in cereals submitted to collaborators by the associate referee this year be adopted as tentative (see p. 87), and that studies on the determination of starch be continued.

(6) That further study be given to the determination of acidity of fat in grain, flour, corn meal, and whole wheat flour and to the correlation of this factor with unsoundness.

(7) That further studies be made of the determination of reducing and nonreducing sugars in flour by the method given in section 20, p. 215, and of its application to the determination of sugar in bread and other cereal products.

(8) That the study of the tentative method for the determination of benzoyl peroxide in flour (44, p. 223) be continued.

(9) That studies be continued on methods for the determination of available carbon dioxide in self-rising flour containing added calcium carbonate.

(10) That methods for the determination of lactose in bread (*This Journal*, 25, 630) be further studied.

(11) That the tentative method for the estimation of milk fat in bread (65, p. 229) be further studied.

(12) That the methods proposed by the associate referee for the determination of proteolytic activity of flour be further studied.

(13) That a study be made of the applicability of methods in the chapter on wheat flour to the determination of moisture, ash, protein, crude fiber, and ether extract in soybean flour.

(14) That studies be made of the detection and determination of soybean flour in cereal products by immunological methods or other suitable means of estimation.

(15) That studies of the determination of added inorganic materials in phosphated and self-rising flours be continued.

(16) That the methods referred to in *This Journal*, 25, 83-84, for the determination of unsaponifiable matter and sterols in noodles be studied to determine their applicability to other foods containing eggs.

(17) That studies of methods for the determination of albumin nitrogen in noodles and other farinaceous egg-containing products be continued.

(18) That methods for the determination of moisture and fat by acid



hydrolysis in fig bars and raisin-filled crackers (*This Journal*, 26, 305) be adopted as tentative and studies continued.

(19) That further studies be made of the modified distillation method (benzene) for the determination of moisture in all flour-like products containing sodium bicarbonate as one of its constituents (*This Journal*, 25, 649).

(21) That methods for the determination of bromates in flour be studied.

(22) That the method for apparent viscosity measurement of flour be studied.

(23) See also Recommendation (14) under Alcoholic Beverages.

#### FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That a collaborative study be made of the reflux method for the determination of peel oil in citrus fruit juices and that the modified oil distillation trap be utilized.

(2) That collaborative work be continued on the method for the determination of beta-ionone where 1 to 10 mg. is present (*This Journal*, 22, 690).

(3) That collaborative studies of the Ripper method for the determination of aldehydes in spirits as applied to lemon oils and extracts (14, p. 174) be continued.

(4) That collaborative studies of the methods proposed by the referee for the determination of esters in lemon extract (*This Journal*, 25, 153) be continued.

(5) That collaborative studies of the Seeker-Kirby method for the determination of esters in lemon and orange oils (U. S. D. A. Bull. 241) be continued.

(6) That collaborative studies of extract mixtures containing both isopropyl alcohol and acetone be continued.

(7) That collaborative study of the photometric method for the determination of vanillin and coumarin (*This Journal*, 25, 155) be continued.

(8) That work be continued on the determination of glycerol, vanillin, and coumarin in vanilla and imitation vanilla extracts with special reference to the automatic extraction of vanillin and coumarin.

(9) That the study of emulsion flavors be continued.

(10) That studies on maple concentrates and imitations be continued.

(11) That a collaborative study of the procedure for the determination of diacetyl (*This Journal*, 24, 655) be made.

#### FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That the tentative colorimetric method for the determination of lactic acid in milk (*This Journal*, 25, 255) be subjected to collaborative study in regard to its applicability to fruit products.

(2) That the Hillig methods for the determination of lactic and volatile fatty acids be subjected to collaborative study.

(3) That methods for the determination of sodium and chlorine be further studied.

(4) That studies of polariscopic methods be continued and that particular reference be given to the possible interference of pectin.

(5) That methods for the determination of total acidity be subjected to collaborative study with the view to adopting a titration procedure with an indicator that will produce results that will correlate with results obtained from electrometric titration.

(6) That the procedures for the determination of polybasic acids published by Hartmann (*This Journal*, 26, 444) be subjected to collaborative study.

(7) That the volumetric procedure for the determination of  $P_2O_5$  published in *This Journal*, 25, 441, be adopted as tentative.

(8) That the tentative colorimetric procedure for the determination of  $P_2O_5$  (39, p. 347) be adopted as official (final action).

(9) That the official gravimetric procedure (38, p. 347) be dropped (first action).

(10) That the gravimetric chloroplatinate procedure for the determination of potassium published in *This Journal*, 26, 326, be adopted as official (first action).

(11) That the gravimetric cobaltinitrite procedure for the determination of potassium published in *This Journal*, 26, 330, be adopted as official (first action).

(12) That the volumetric chloroplatinate method (*This Journal*, 25, 232); the short gravimetric procedure (*Ibid.*, 26, 473 and 25, 434); and also the short volumetric procedure (*Ibid.*, 26, 324) be adopted as tentative.

(13) That the unified procedures for the determination of potassium published in *This Journal*, 26, 324, be subjected to collaborative study.

(14) That further work be done on methods for sampling cold pack fruit.

#### SUGAR AND SUGAR PRODUCTS

It is recommended—

(1) That studies on unfermented reducing substances in molasses be continued.

(2) That collaborative study of the procedure for the determination of diacetyl (*This Journal*, 24, 655) be referred to the Referee on Flavors and Nonalcoholic Beverages.

(3) That studies on the determination of moisture in liquid sugar products be continued.

(4) That the tentative method for the determination of dextrin in honey (92, p. 510) be studied as suggested by the associate referee.

(5) That the official method for the determination of free acid in honey (93, 510) be studied with a view to making it more definite with respect to the end point of the titration.

(6) That study of the double alcoholic precipitation method (*This Journal*, 25, 683) for determination of dextrin in honey be discontinued, and that other methods for this determination be studied.

(7) That the determination of moisture in honey by means of a refractometer and the table of refractive index and moisture equivalents of H. D. Chataway (*This Journal*, 25, 99) be adopted as official (final action).

(8) That the methods for the determination of ash in sugar and sugar products (9, 10, p. 487) be changed to provide for the use of a sample of appropriate weight for the product being examined.

(9) That studies on confectionery be continued.

(10) That Ofner's method for the determination of small quantities of invert sugar in refined sugars, as modified by Jackson and McDonald (*This Journal*, 26, 462), be subjected to collaborative work with a view to its adoption to replace Herzfeld's Method I (45, p. 503 with supplementing Table 10, p. 677).

(11) That the Hagedorn and Jensen micro method for the determination of small quantities of invert sugar in refined sugars (*Biochem. Z.*, 135, 46 (1923)) be studied.

(12) That Hammond's copper equivalents of dextrose, levulose, invert sugar, and mixtures of invert sugar and sucrose containing, respectively, 0.3, 0.4, and 2.0 grams of total sugar (NBS Research Paper RPI301, p. 589; *J. Research Nat. Bur. Standards*, 24, 579 (1940)), be adopted as official (first action).

(13) That the study of available methods for the analysis of corn sirups, corn sugars, and allied products be continued.

(14) That methods of color measurement in solutions of sugar products be studied.

(15) That a method for preparation of standard invert sugar solution be studied.

(16) That the work on the determination of invert sugar in mixtures of sucrose and invert sugar by Munson and Walker's method be continued with a view to replacing the Meissl and Hiller table of factors.

#### WATERS, BRINE, AND SALT

It is recommended—

(1) That the tentative method of reporting results on waters and brine (81, p. 543) and the table of combining weights and their reciprocals (82, p. 544) be made official (first action). These figures were calculated from the International Atomic Weights of 1939, but no change was noted in the 1943 table.

(2) That studies on methods for the determination of boron in waters be continued.

(3) That the method for the determination of fluorides in water (22, p. 529; corrected in *This Journal*, 25, 101) be made official (final action).

(4) That methods for the determination of fluorine in salt be further studied.

(5) That methods for the determination of iodides in salt (58, p. 368; *This Journal*, 26, 440; Sadusk-Ball, *Ind. Eng. Chem., Anal. Ed.*, 5, p. 386) be studied collaboratively.

(6) That the Elmslie-Caldwell procedure for the determination of iodine in mineral mixed feed (58, p. 368), as modified by the Associate Referee on Salt (see p. 110) be made official (first action) for the determination of iodine in salt.

(7) That tentative methods I and II for the determination of iodides in salt (100-105, pp. 547 and 548) be discontinued.

(8) That the tentative procedure for the preparation of samples of salt (113, p. 549) and the tentative method for the determination of sulfates in salt (118, p. 550) be further studied.

(9) That methods for sampling iodized salt be further studied.

## CHANGES IN OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE FIFTY-EIGHTH ANNUAL MEETING, OCTOBER 27, 28, 1943\*

### I. SOILS

No additions, deletions, or other changes.

### II. FERTILIZERS

(1) For sampling fertilizers the slotted single-tube sampler, the slotted double-tube sampler, and the slotted tube and rod sampler, all with solid pointed ends (*This Journal*, 24, 501), were adopted as official (final action).

(2) The use of a factor weight was provided in the official method for the determination of potash by the addition to line 1, 41(a), p. 31, and to line 2 of 41(b), of the following directions: "or the factor weight 2.425 g," and to 42(a), the following sentence was added: "If the factor weight and a 50 ml aliquot (containing 0.485 g of sample) are used, multiply weight by 40 to obtain per cent  $K_2O$ " (final action).

(3) The official method for the determination of water-insoluble organic nitrogen (34, p. 29) was deleted (final action).

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\* Compiled by Marian E. Lapp, *Associate Editor*. Unless otherwise given all references in this report are to *Methods of Analysis*, A.O.A.C., 1940, and the methods are edited to conform to the style used in that publication.

(4) For section (b) of the official method for the determination of nitrate nitrogen (31, p. 28) there was substituted the following: "Determine water-insoluble organic nitrogen as directed under 35, but use 2.5 g of the mixed fertilizer" (official, final action).

(5) The heading "Total Nitrogen," preceding paragraphs 24, 25, 26, and 27, was changed to read, "Total Nitrogen in Mixed Fertilizers," (official, final action).

(6) The words "in mixed fertilizers" were added to the heading "Nitrate Nitrogen," preceding par. 31, p. 28 (official, final action).

(7) In line 6 of par. 60, p. 38, after the words "for unmixed nitrate salts," the words "or for mixed fertilizers containing considerable nitrate nitrogen" were added (official, final action).

(8) The long volumetric method for the determination of copper, adopted as official (first action) in 1941 and published in *This Journal*, 24, 67, was adopted as official (final action).

(9) The statement "(Not applicable to samples containing free ammonia or compounds other than water that are volatilized at the temperature of drying)" was added to the method for the determination of moisture by drying (4, p. 20) (official, final action).

(10) The paragraph relating to graduated flasks, published in *This Journal*, 25, 78, following Recommendation 17, was added to Chapter II (official, final action).

(11) In the official Devarda method (30, p. 27) the quantity "0.5 g" was changed to "0.35 or 0.5 g"; the last paragraph was changed to read "In the analysis of nitrate salts, dissolve 3.5 or 5.0 g in  $H_2O$ , make up to 250 ml, and use 25 ml"; and the same sentence was added to the ferrous sulfate-zinc-soda method for the same determination (29, p. 27) (official, final action).

(12) The following sentence was added to the method for the preparation of standard sodium or potassium hydroxide solution (10(b), p. 22): "Burets in constant use are likely to become so corroded as to increase their capacity, and therefore should be tested at least once a year" (official, final action).

(13) The following clarifying paragraph was added to the method for the determination of potash in nitrate of potash, as 41(e), p. 31: "*Nitrate of potash or nitrate of potash and soda.*—If impure, proceed as directed in (a); if sufficiently pure, proceed as directed for potash salts (b), except to evaporate an aliquot to dryness in a porcelain dish with 2 ml of HCl (if Pt dish is used, add  $H_2SO_4$  instead) and to take up with  $H_2O$  and a few drops of HCl, before adding the Pt soln" (official, final action).

(14) Method III, volumetric modification of the method for the determination of acid-soluble magnesium (36, p. 54), adopted as official (first action), (*This Journal*, 24, 47) was adopted as official (final action).

(15) The bismuthate method for the determination of acid-soluble

manganese (*This Journal*, 24, 69), adopted as official (first action), *Ibid.*, 25, 49, was adopted as official (final action).

(16) The official (first action) volumetric periodate method for the determination of manganese (56, 57, p. 37) was deleted (final action).

(17) The following colorimetric method for the determination of acid-soluble manganese in fertilizers (58, p. 37; *This Journal*, 24, 47; 25, 49, 79) was adopted as official (final action):

**ACID-SOLUBLE MANGANESE**

*Colorimetric Method for Fertilizers*

(Applicable to samples with not more than 5% manganese)

Place 1 g of sample in 200 ml wide-neck volumetric flask or a 200 ml beaker. Add 10 ml of  $\text{H}_2\text{SO}_4$  and 30 ml of  $\text{HNO}_3$ . Heat gently until brown fumes diminish, then boil 30 min. If organic matter is not destroyed, cool, add 5 ml of  $\text{HNO}_3$ , and boil. Repeat this process until no organic matter remains, and boil until white fumes appear. Cool slightly, and add 50 ml of  $\text{H}_3\text{PO}_4$  soln (90 ml of  $\text{H}_2\text{O}$ , 10 ml of 85%  $\text{H}_3\text{PO}_4$ ). Boil for a few minutes. Cool, make to 200 ml in a volumetric flask, mix, and let stand to allow precipitation of  $\text{CaSO}_4$ . Pipet 50 ml of clear soln into a beaker. Heat nearly to boiling point, with stirring or whirling add 0.3 g of  $\text{KIO}_4$  for each 15 mg of Mn present, and proceed as directed in XXVII, 60. At final dilution soln should contain not more than 200 p.p.m. of Mn. Calculate to Mn.

(18) Method I, official, for the determination of acid-soluble magnesium (52, p. 35) was deleted, and the parts of this method referred to in Method II (53, p. 36) were incorporated in Method II (official, first action).

(19) The following directions for titration with a glass electrode in the method for the determination of acid- and base-forming quality, were adopted as tentative:

Proceed as directed in 60, through the addition of 50 ml of water and 30 ml of normal HCl and digestion on a hot plate or steam bath for 1 hour. Cool to room temperature and without filtering titrate the soln in the 150 ml beaker with 0.5 N NaOH to pH 4.3, using a glass electrode apparatus or other standard means of electrometric titration, and a continuous stirrer. Make the usual blank titration, using the glass electrode. Calculate results as directed in 60.

(20) The methods applicable to basic slag were revised as follows to obviate a separate section: Delete 61. Change heading of 3 to read "Mechanical analysis of bone, tankage, and basic slag" and add to method, "With basic slag, proceed as directed in 8(b)." Delete 62 and 63. Add to 8: "With basic slag proceed as directed in 8(b)." Delete 64 and add to 9 the following directions: "With basic slag dehydrate aliquot (20 ml) of prepared soln by evaporating to dryness on steam or hot water bath. Treat with 5 ml of HCl and 25 ml of hot  $\text{H}_2\text{O}$ , digest in order to complete the soln, and filter off  $\text{SiO}_2$ . Proceed as directed above. Before precipitating with magnesia mixture, add 5 ml of 5% Na acetate soln." Delete 65, and add this paragraph to 12, as (c), preceded by the words, "In basic slag." Run the material in 66, 67, and 68 immediately after 17, with the heading "Citrate Acid-soluble Phosphoric Acid in Basic Slag—Official."

## III. SEWAGE\*

## IV. AGRICULTURAL LIMING MATERIALS

The following method for the determination of neutralizing value of calcium silicate slags was adopted as tentative:

## NEUTRALIZING VALUE OF CALCIUM SILICATE SLAGS

(a) *Blast Furnace Slags*.—Weigh .5 g charge ground to pass 80-mesh sieve and transfer into a 250 ml Erlenmeyer flask. Wash down with small portions of  $H_2O$  and introduce 35 ml of .5 *N* HCl while swirling. Heat to a gentle boil over Bunsen burner, *agitating the suspension continuously* until the bulk of sample has dissolved. Maintain boiling for 5 min.; cool to room temp., transfer to 150 ml beaker, and dilute to ca 80 ml. Titrate with .5 *N* NaOH to pH 4.6 as determined by a glass electrode. Net ml of acid used  $\times 5$  = neutralizing value of the slag in terms of  $CaCO_3$ .

(b) *Slags from Rock Phosphate Reduction Furnaces*.—Weigh .5 g charge and transfer into 250 ml beaker. Wash down with small portions of  $H_2O$  and introduce 50 ml of acetic acid (1+4). Stir suspension continuously during addition of acid. Heat to boiling 5 min., stirring frequently. Evaporate to a gel on hot plate or sand bath. Add 40 ml of acetic acid (1+4), dilute to 150 ml, and heat to boiling; add  $NH_4OH$  (1+1) to clear yellow of methyl red and distinct odor of  $NH_4$ . Digest ca 15 min. on hot plate. Filter by gravity on 9 cm paper, catching filtrate in a 220 ml shallow-form porcelain dish; wash beaker 3 times and the filter 5 additional times with neutral .5 *N*  $NH_4$  acetate. Evaporate filtrate on hot plate. To prevent spattering adjust heat so that the bubbles that break through the viscous surface film are released gently. (Dehydration may be expedited by 2 or 3 repeated treatments with 25 ml of hot  $H_2O$  and evaporation.) Continue heating residue on hot plate until odor of acetic acid can not be detected. Heat an additional 10 min. at full heat of hot plate and then ignite 10 min. in electric furnace at  $550^\circ$ . Cool, wet residue with 15 ml of  $H_2O$ , place cover-glass over dish, and introduce 25 ml of .5 *N* HCl through lip of beaker. Heat 5 min. over Bunsen burner to gentle simmer. Rinse cover-glass; filter suspended Mn oxides on 9 cm filter, catching filtrate in 250 ml Erlenmeyer flask; and wash dish and filter 3 times with hot  $H_2O$ . Titrate excess acid with .5 *N* NaOH to clear yellow of methyl red. Net acid used  $\times 5$  = neutralizing value of the slag in terms of  $CaCO_3$ .

## V. AGRICULTURAL DUST\*

## VI. INSECTICIDES AND FUNGICIDES

(1) The value, 1 ml (M/100)  $KIO_3$  = 5.70 mg of Pyrethrum I, was adopted as the factor in the mercury reduction method for Pyrethrin I in pyrethrum powder and extracts (115, p. 67), (official, first action).

(2) The following procedures were adopted as substitutes for the official lead chlorofluoride method for the determination of total fluorine (18, p. 49), (official, first action).

## TOTAL FLUORINE

## REAGENTS

(a) *Fusion mixture*.—Mix anhydrous  $Na_2CO_3$  and  $K_2CO_3$  in equimolecular proportions.

(b) *Lead chlorofluoride wash soln.*—Dissolve 10 g of  $Pb(NO_3)_2$  in 200 ml of  $H_2O$ ; dissolve 1 g of NaF in 100 ml of  $H_2O$  and add 2 ml of HCl; and mix these 2 solns. Allow precipitate to settle and decant supernatant liquid. Wash 4 or 5 times with 200

\* Subject for future study.

ml of  $H_2O$  by decantation, and then add ca 1 liter of cold  $H_2O$  to precipitate and allow to stand 1 hour or longer, with occasional stirring. Pour through filter and use clear filtrate. By adding more  $H_2O$  to the precipitate of  $PbClF$  and stirring, more wash soln may be prepared as needed.

(c) *Standard silver nitrate soln.*—0.1 or 0.2 *N*. Standardized by titration against pure  $NaCl$ , using  $K_2CrO_4$  indicator.

(d) *Standard potassium or ammonium thiocyanate soln.*—0.1 *N*. Standardize by comparing with the standard soln of  $AgNO_3$  under the same conditions as obtained in the determination.

(e) *Ferric indicator.*—Add to cold saturated soln of ferric alum (free from  $Cl$ ) sufficient colorless  $HNO_3$  to bleach the brown color.

(f) *Bromophenol blue indicator.*—Grind 0.1 g of the powder with 1.5 ml. of 0.1 *N*  $NaOH$  soln and dilute to 25 ml.

#### DETERMINATION

(1) *Samples difficult to decompose such as cryolite; and others that contain aluminum or appreciable quantities of silicious material.*—Mix 0.5 g of sample (or less if necessary to make content of  $F$  0.01 to 0.10 g) with 5 g of fusion mixture and 0.2–0.3 g of powdered silica, cover with 1 g of fusion mixture, and heat to fusion over Bunsen burner. (Use of blast lamp is not required as it is only necessary that the mass be fluid, and it is preferable not to heat much beyond the temp. at which it melts. If much  $Al$  is present, a uniform, clear, liquid melt cannot be obtained. There will be particles of a white solid separated in the liquid. The melt after cooling should be colorless, or at least should not have more than a gray color.)

Leach cooled melt with hot  $H_2O$  and filter into a 400 ml beaker when disintegration is complete. Return insoluble residue to Pt dish by use of jet of  $H_2O$ , add 1 g of  $Na_2CO_3$ , make to volume 30–50 ml, boil for a few minutes, disintegrating any lumps with glass rod flattened on end, filter through same paper, wash thoroughly with hot  $H_2O$ , and adjust volume of filtrate and washings to ca 200 ml. Add 1 g of  $ZnO$  dissolved in 20 ml. of  $HNO_3$  (1+9), boil 2 min. with constant stirring, filter, and wash thoroughly with hot  $H_2O$ . During this washing return the gelatinous mass to beaker three times and thoroughly disintegrate in the wash soln because it is difficult to wash this precipitate on filter. (The mass can easily be returned to beaker by rotating funnel above beaker and at the same time cutting precipitate loose from paper with jet of wash soln.)

Add 2 drops of bromophenol blue, and with a cover-glass almost over the beaker add  $HNO_3$  (1+4) until color just changes to yellow. Make soln slightly alkaline with 10%  $NaOH$ , and with cover-glasses on the beakers boil gently to expel  $CO_2$ . Remove from burner, add the  $HNO_3$  until color just changes to yellow and then the dilute  $NaOH$  until color just changes to blue, and add 3 ml of 10%  $NaCl$ . (Volume of soln at this point should be 250 ml.)

Add 2 ml of  $HCl$  (1+1) and 5 g of  $Pb(NO_3)_2$ , and heat on steam bath. As soon as the  $Pb(NO_3)_2$  is in soln, add 5 g of  $Na$  acetate, stir vigorously, and digest on steam bath 30 min. with occasional stirring. Allow to stand overnight, and wash precipitate, beaker, and paper once with cold  $H_2O$ , then 4 or 5 times with a cool saturated soln of  $PbClF$  and then once more with cold  $H_2O$ .

Transfer precipitate and paper to beaker in which precipitation was made, stir paper to pulp, add 100 ml of  $HNO_3$  (5+95), and heat on steam bath until precipitate is dissolved. (5 min. is ample to dissolve this precipitate. If sample contains an appreciable quantity of sulfates the precipitate will contain  $PbSO_4$ , which will not dissolve. In such a case heat 5–10 min. with stirring and consider the  $PbClF$  to be dissolved.) Add a slight excess of 0.1 *N* or 0.2 *N*  $AgNO_3$  soln, digest on steam bath 30 min., cool to room temp. while protected from light, filter, wash with cold  $H_2O$ ,



and determine  $\text{AgNO}_3$  in the filtrate by titration with the standard thiocyanate soln, using 10 ml of the ferric indicator. Subtract quantity of  $\text{AgNO}_3$  found in the filtrate from that originally added. The difference will be that required to combine with the Cl in the  $\text{PbClF}$ , and from this difference calculate percentage of F in sample on basis that 1 ml of 0.1 N  $\text{AgNO}_3$  = 0.0019 g of F or 0.2 N  $\text{AgNO}_3$  = 0.00380 g of F.

(2) *Water-soluble fluorides in presence of up to 50% organic matter such as flour, pyrethrum, tobacco powder, and derris or cube powders, which are readily decomposed without addition of powdered silica and free from or contain only small quantities of aluminum or silicious compounds.*—Mix 0.5 g (or less if necessary to make content of F fall between 0.01 and 0.1 g) of sample with 5 g of fusion mixture, cover with 1 g of fusion mixture, and heat to fusion over Bunsen burner. Leach cooled melt with hot water, filter into a 600 ml beaker when disintegration is complete, and wash thoroughly with hot  $\text{H}_2\text{O}$ . Proceed as directed under (1), beginning "Add 2 drops of bromophenol blue, and with cover-glass almost over the beaker add  $\text{HNO}_3$  (1+4) until the color just changes to yellow."

(3) *Water-soluble samples in absence of organic matter and appreciable quantities of sulfates or aluminum salts.*—In the absence of organic matter or other interfering substances the fusion may be omitted and determination made on an aliquot of a water-soluble soln containing between 0.01 and 0.1 g of F, as directed in (1), beginning "Add 2 drops of bromophenol blue."

In the presence of aluminum, as in samples containing sodium silicofluoride and potassium aluminum sulfate, transfer sample to 400 ml beaker, dissolve in 150 ml of hot water, add 6 g of the fusion mixture, and boil. Add 1 g of  $\text{ZnO}$  dissolved in 20 ml of  $\text{HNO}_3$  (1+9), boil 2 min. with constant stirring, filter into a 500 ml volumetric flask, and wash thoroughly with hot  $\text{H}_2\text{O}$ . Cool to room temp. and make to volume. Transfer a 200 ml aliquot containing 0.01–0.10 g of F to a 600 ml beaker and proceed as directed in (1), beginning "Add 2 drops of bromophenol indicator."

(4) *Sodium and magnesium silicofluorides in absence of aluminum and boron, with or without organic matter.*—With large quantities of sodium silicofluoride and some of the more volatile silicofluorides, for example magnesium, where there is a possibility of some of the F being evolved as  $\text{SiF}_4$  before the melt with the fusion is effected, distil the F as directed in 23, p. 51, and determine F in distillate. Add several drops of bromophenol blue, make alkaline with  $\text{NaOH}$ , and adjust volume to ca 250 ml by gently boiling volume down from 400 to 260 ml. Proceed as directed under (1), beginning "Remove from burner, add  $\text{HNO}_3$  (1+4)."

(5) *Samples containing large quantities of organic matter or appreciable quantities of sulfates.*—Proceed as directed in (4). If sample contains over .8 g of organic matter, make distillate alkaline with  $\text{NaOH}$ , evaporate to 25–30 ml, and re-distil.

NOTES: These procedures give accurate results for quantities of F between 0.01 and 0.10 g. Below 0.01 g the results have a tendency to be slightly low and above 0.10 slightly high. A convenient sample to fuse is one that contains 0.07–0.08 g of F, and it is inadvisable to use too large a sample as an incomplete fusion may result. Large quantities of boron compounds and alkali salts retard or prevent the complete precipitation of lead chlorofluoride. Boron has a greater effect when the quantity of F is large than when it is small. In the procedures described boron has little effect, and it may be disregarded in the analysis of insecticides if the quantity of F to be precipitated is not more than 0.03 g. With some compounds containing borax or boric acid, where it is difficult to obtain a representative mixture when an extremely small sample (0.1 g) is used for analysis, a larger sample may be taken and the lead chlorofluoride precipitated from an aliquot of the fusion soln. The quantity of alkali carbonates specified in the fusion and in washing of the insoluble residue is not large enough to cause low results. If sample contains S, it should be removed with  $\text{CS}_2$ , and F determined on air-dry residue, allowance being made in calculations for percentage of S removal.

## VII. CAUSTIC POISONS

No additions, deletions, or other changes.

## VIII. NAVAL STORES

No additions, deletions or other changes.

## IX. PAINTS, VARNISHES AND CONSTITUENT MATERIALS

No additions, deletions, or other changes.

## X. LEATHERS

No additions, deletions, or other changes.

## XI. TANNING MATERIALS

No additions, deletions, or other changes.

## XII. PLANTS

The following methods for the determination of chlorophyll were adopted as tentative:

## CHLOROPHYLL

*Photoelectric Colorimetric Method for Total Chlorophyll Only*<sup>1,2</sup>

## 1

## APPARATUS

(a) *Filter paper*.—A good grade or quantitative paper to fit the Büchner funnels if used.

(b) *Flasks*.—Suction flasks of 500 ml capacity and volumetric flasks of 100–500 ml capacity.

(c) *Funnels*.—Small Büchner funnels or sintered glass funnels of medium porosity.

(d) *Mortar and pestle*.—A deep glass mortar ca 4" in diameter, with a well-defined lip, is recommended.

(e) *Photoelectric colorimeter*.—Calibrate for chlorophyll by means of a plant extract as directed in 3, using light filters that give maximum light transmission near 6600 Å. (A combination of Corning H. R. light filters Nos. 243 and 396 is suitable for this purpose.)

(f) *Wash bottles*.—The type fitted with a rubber bulb, which permits operation with one hand, is recommended.

(g) *Waring blender or similar machine*.—Vessels similar to No. 3, shown in *This Journal*, 25, 583, possess advantages over the original blender container.

(h) *Wiley mill or similar grinding machine*.—If dried samples are to be analyzed.

(i) *Hand shears or scissors and rubber policeman*.

## 2

## REAGENTS

(a) *Acetone*.—Undiluted acetone and 85% soln by vol. Commercial acetone of technical grade is satisfactory.

(b) *Calcium or sodium carbonate*.—C. P. grade.

(c) *Quartz sand*.—Acid-washed and dried.

## 3

## DETERMINATION

Select field material carefully to insure a representative sample. Remove a representative portion from field sample, and if fresh material, cut finely with hand

<sup>1</sup> Petering, Wolman, and Hibbard, *Ind. Eng. Chem., Anal. Ed.*, 12, 148 (1940).

<sup>2</sup> Comar, Benne, and Buteyn, *Ibid.*, 15, 524 (1943).

shears and mix as thoroughly as possible. Grind dried material in a mill and mix thoroughly. Weigh 1-5 g into the mortar and add a small quantity (ca 0.1 g) of  $\text{CaCO}_3$  or  $\text{Na}_2\text{CO}_3$ . Macerate tissue with pestle, add the quartz sand, and grind for a short time; then add the 85% acetone soln, a little at a time, and continue grinding until tissue is finely ground. Transfer mixture to funnel, draw off soln with suction, and wash residue with the 85% acetone soln. Return residue to mortar with more acetone soln and grind again. Filter, and wash as before. Repeat this procedure until tissue is devoid of green color and washings are colorless. (It is advisable to grind the residue at least once with undiluted acetone and then to add sufficient  $\text{H}_2\text{O}$  at the end to bring it to 85% soln. A Waring blender or similar machine may be used for macerating and extracting the tissue instead of the mortar (see 6), but each investigator should satisfy himself that complete extraction of the tissue is accomplished by the device used.) When extraction is complete, transfer filtered extract to volumetric flask of appropriate size and make to volume.

Measure the percentage of light transmitted by the soln with a photoelectric colorimeter that has been calibrated for chlorophyll by use of a plant extract as described below. Read the amount of chlorophyll present from the curve relating light transmission and concentration. Express chlorophyll values as mg/g of tissue, or in other convenient manner.

Calibration of the photoelectric colorimeter is accomplished as follows: Extract a sample of fresh, green leaf material with the 85% acetone soln, filter, wash residue, and make extract to volume as directed above. Make a series of dilutions of the extract and measure the % of light transmitted by the original and by each of the diluted solutions with the instrument in the same manner as when a chlorophyll preparation is being used as the calibration standard. Transfer an aliquot of the original extract to diethyl ether and evaluate total chlorophyll by the spectrophotometric method as directed in 6 (b) and (c). From the value thus obtained calculate the chlorophyll content of the original extract and that of each of the diluted solns, and construct the curve relating concentration of chlorophyll and % of light transmitted, or the  $\log_{10} I_0/I$  values, as usual.

#### *Spectrophotometric Method for Total Chlorophyll and the a and b Components<sup>3,4</sup>*

##### APPARATUS

The apparatus listed under 1 with the exception of the photoelectric colorimeter and Wiley mill, plus the following:

(a) *Bottles*.—Small reagent bottles of ca 60 ml capacity, with either glass or cork stoppers, are convenient receptacles in which to bring the diethyl ether solns to the proper dilution.

(b) *Pipets*.—Volumetric pipets of various capacities are required for making necessary dilutions, also straight dropping pipets (medicine droppers).

(c) *Scrubbing tubes for washing ether solns*.—Open tube of ca 20 mm diameter, to one end of which is sealed a tube of smaller diameter drawn to a fine jet at the lower end.

(d) *Separatory funnels*.—250-500 ml capacity.

(e) *Double-deck separatory funnel support*.

(f) *Spectrophotometer*.—Must be capable of isolating a spectral region of ca 30 Å near 6600 Å with negligible stray radiation. Tubulated cells with tightly fitting glass stoppers are recommended for work with diethyl ether.

<sup>3</sup> Comar, C. L., *Ind. Eng. Chem., Anal. Ed.*, 14, 877 (1942).

<sup>4</sup> Comar and Zscheile, *Plant Physiology*, 17, 198 (1942).

## 5

## REAGENTS

Those listed under 2 plus the following:

(a) *Diethyl ether*.—Commercial ether designated as purified for fat extraction is satisfactory without further purification.

(b) *Sodium sulfate*.—C. P. grade, anhydrous.

(c) *Trisodium phosphate*.—Glassware should be washed with a strong soln of this salt in order to remove traces of acid that might tend to decompose chlorophyll.

## 6

## DETERMINATION

(a) *Extraction of chlorophyll from tissue*.—Select and prepare sample as directed in 3. Disintegrate a weighed portion (2–10 g, depending upon chlorophyll content) of fresh plant tissue in a Waring blender cup, which contains a small quantity (ca 0.1 g) of  $\text{CaCO}_3$ , or by use of a mortar as described in 3. After the tissue is thoroughly disintegrated, filter extract through a Büchner funnel fitted with a quantitative filter paper. Wash residue with the 85% acetone soln, and if necessary use a little diethyl ether to remove the last traces of pigment. If extraction is incomplete, return residue and filter paper to blender container with more acetone soln and repeat extraction. Filter, and wash as directed previously into flask containing the first filtrate. Transfer filtrate to volumetric flask of appropriate size and make to volume with the 85% acetone. Pipet an aliquot of 25–50 ml into a separatory funnel containing ca 50 ml of diethyl ether. Add  $\text{H}_2\text{O}$  carefully until it is apparent that all the fat-soluble pigments have entered the ether layer. Draw off and discard the water layer. Place the separatory funnel containing the ether soln in the upper rack of the support. Add ca 100 ml of  $\text{H}_2\text{O}$  to a second separatory funnel and place it in the rack below the first. Set the scrubbing tube in place and allow ether soln to run through it to bottom of lower funnel and rise in small droplets through the  $\text{H}_2\text{O}$ . When all soln has left upper funnel, rinse it and scrubbing tube with a little ether added from a medicine dropper. Place scrubbing tube in upper funnel and exchange its place in the support with the funnel now containing the ether soln. Draw off and discard the  $\text{H}_2\text{O}$  in upper funnel, add a similar portion of fresh  $\text{H}_2\text{O}$  to lower funnel, and repeat washing process. Continue washing ether soln until all acetone is removed (5–10 washings). Then transfer ether soln to 100 ml volumetric flask, make to volume, and mix.

(b) *Spectrophotometric measurements*.—Add ca teaspoonful of anhydrous  $\text{Na}_2\text{SO}_4$  to 60 ml reagent bottle, and fill it with the ether soln of the pigment. When this soln is optically clear, pipet an aliquot into another dry bottle and dilute it with sufficient dry ether to cause the  $\log_{10} I_0/I$  value to fall between 0.1 and 0.8 at wave length to be used. The most favorable value is near 0.6 at 6600 Å, since such a soln will yield a satisfactory value at 6425 Å. Fill two clean glass-stoppered absorption cells with dry ether by use of pipet, and polish outside surfaces of each, first with cotton wet with ethanol and then with dry cotton. Place cells in instrument, and determine whether each gives the same galvanometer deflection. If not, clean again or select cells that do, and do this daily. Empty one cell, fill it with the dried ether soln, and place in instrument. Adjust entrance and exit slits until spectral region isolated is 30–40 Å at 6600 Å. Determine whether instrument is in proper adjustment for wave length by taking readings through the solvent and the soln at intervals of 10 Å from 6580–6650 Å. Calculate the  $\log_{10} I_0/I$  value for each wave length at which readings were taken. The highest value should occur at 6600 Å; if it does not, adjust machine until it does or make the 6600 Å readings at the wave length setting that gave the highest value. In the case of a grating instrument apply the same correction at 6425 Å; however, with a prism instrument the correction at 6425 Å must be obtained from a wave-length calibration curve for the particular instrument in use. Calibrate instrument for wave length in this way often enough to insure that it remains in

proper adjustment. Take  $I_0$  and  $I$  readings at 6600 and 6425 Å (or the corrected settings) for each unknown soln.

(c) *Calculation of chlorophyll concentration.*—Calculate the  $\log_{10} I_0/I$  values for each of the readings made, substitute them in the following simplified equations, and solve for total chlorophyll and each of the  $a$  and  $b$  components as follows:

$$(1) \text{ Total chlorophyll (mg/l) } = 7.12 \log_{10} \frac{I_0}{I} \text{ (at 6600 Å) } + 16.8 \log_{10} \frac{I_0}{I} \text{ (at 6425 Å).}$$

$$(2) \text{ Chlorophyll } a \text{ (mg/l) } = 9.93 \log_{10} \frac{I_0}{I} \text{ (at 6600 Å) } - 0.777 \log_{10} \frac{I_0}{I} \text{ (at 6425 Å).}$$

$$(3) \text{ Chlorophyll } b \text{ (mg/l) } = 17.6 \log_{10} \frac{I_0}{I} \text{ (at 6425 Å) } - 2.81 \log_{10} \frac{I_0}{I} \text{ (at 6600 Å).}$$

#### SUPPLEMENTARY INFORMATION

The factors involved in the spectrophotometric analysis of the chlorophyll system have been discussed in detail by Comar and Zscheile.<sup>4</sup> These authors used Beer's law in the form:

$$c = \frac{\log_{10} \frac{I_0}{I}}{\alpha l}, \quad \text{where}$$

$I_0$  is intensity of light transmitted by solvent-filled cell,

$I$  is intensity of light transmitted by soln-filled cell,

$c$  is concentration of chlorophyll (g/liter),

$\alpha$  is specific absorption coefficient, and

$l$  is thickness of solution layer in cm.

Since at a given wave length the observed  $\log_{10} I_0/I$  value of a soln having two components represents the sum of the  $\log_{10} I_0/I$  values of each of the components, the following equation obtains in the case of chlorophyll  $a$  and  $b$  at a given wave length:

$$(4) \quad \left( \log_{10} \frac{I_0}{I} \right)_{\text{observed}} = \left( \log_{10} \frac{I_0}{I} \right)_a + \left( \log_{10} \frac{I_0}{I} \right)_b.$$

If a 1 cm cell is used this equation may be expressed as:

$$(5) \quad \left( \log_{10} \frac{I_0}{I} \right)_{\text{observed}} = \alpha_a c_a + \alpha_b c_b.$$

The concentrations of chlorophylls  $a$  and  $b$  in a given diethyl ether soln can now be calculated by the use of equation (5) as follows:

(a) Determine  $\log_{10} I_0/I$  values for the soln at two different wave lengths (6600 and 6425 Å have been found advantageous for this purpose).

(b) Select proper specific absorption coefficients corresponding to the wave lengths used from the table.

(c) Substitute the observed  $\log_{10} I_0/I$  value and the specific absorption coefficient in equation (5) for each of the two wave lengths used as illustrated for 6600 and 6425 Å in equations (6) and (7). Solve these two equations simultaneously for the two unknowns, the concentrations of chlorophylls  $a$  and  $b$ .

$$(6) \quad \log_{10} \frac{I_0}{I} \text{ (at 6600 Å) } = 102c_a + 4.50c_b.$$

$$(7) \quad \log_{10} \frac{I_0}{I} \text{ (at 6425 Å) } = 16.3c_a + 57.5c_b.$$

Equations (1), (2), and (3) were derived in this way.

The criterion for the accuracy of the chlorophyll values as determined by the spectrophotometric method is the agreement between analytical results as determined from measurements at different wave lengths. It has been demonstrated by Comar & Zscheile<sup>4</sup> that measurements at 6600 and 6425 Å are convenient for routine analysis; however, readings may be made at other wave lengths to check these values. Specific absorption coefficients for chlorophyll *a* and *b* in diethyl ether soln that may be used for this purpose are presented in the following table.

*Absorption constants used in analysis*  
(After Comar and Zscheile<sup>4</sup>)

Wave length Å	Specific absorption coefficients (For diethyl ether solns)	
	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>
6600	102.	4.50
6425	16.3	57.5
6000	9.95	9.95
5810	8.05	8.05
5680	7.11	7.11
6130	15.6	8.05
5890	5.90	10.3

These values may be used for calculations as follows:

(a) Values for total chlorophyll and percentage composition may be calculated from absorption values at 6600 and 6425 Å as described.

(b) Check values for total chlorophyll may be calculated from the absorption values at the intersection points 6000, 5810 and 5680 Å.

(c) Check values for percentage composition may be calculated from the absorption values for each of the points 6130 and 5890 Å in combination with a value of total concentration as obtained from (a) or (b).

### XIII. BEVERAGES (NONALCOHOLIC) AND CONCENTRATES

No additions, deletions, or other changes.

### XIV. MALT BEVERAGES, SIRUPS AND EXTRACTS, AND BREWING MATERIALS

(1) The following method for the determination of bushel weight was adopted as an alternative tentative method, but it is not to be used to determine bushel weight for the purpose of determining compliance with the requirement of the U. S. Grain Standards Act.

#### BUSHEL WEIGHT

Weigh 110 g of the sample to the nearest 0.1 g and pour evenly into a metal funnel provided with a plunger discharge and placed on top of a 250 ml Normax cylinder graduated to meet N.B.S. specifications. (The funnel must fit snugly into the graduate and be sufficiently large to accommodate the grain without danger of spilling when the plunger is raised.) Then drop the material into the cylinder by pulling plunger up. Do not jar or tap the cylinder during this operation or before reading the volume, and do not read the very upper grain level, as compensation must be made for the ends of the few kernels that protrude. If the grain surface has a slant, repeat the test.

Make the calculation, which is based on the consideration that the Winchester bushel used in this country holds 2150.42 cu. in. or 35.239 ml, as follows:

$X = W/Y$ , where  $X$  represents bu. wt. in lbs.,  $Y$  the volume in Winchester bushels, and  $W$  the weight in lbs. If weight is constant ( $K$ ),  $X = K/Y$  or  $XY = K$ . To ascertain  $K$ , let bushel weight equal unity, then with the constant weight of sample in the test 110 g—

$$\frac{1 \text{ lb.}}{1 \text{ bu.}} = \frac{453.6 \text{ g}}{35.239 \text{ ml}} = \frac{110 \text{ g}}{8545 \text{ ml}} \cdot \text{Therefore } K = 8545 \text{ and bushel weight in lbs.} \\ = \frac{8545}{\text{volume occupied by 110 g sample in ml}} \cdot$$

(2) The methods for the analysis of caramel malt and black malt published in *This Journal*, 26, 300–301, were adopted as tentative.

(3) The ferricyanide modification for the determination of reducing sugars after diastasis was adopted as a tentative optional alternative procedure in the method for the determination of diastatic power (48, p. 161). Paragraph 48 has been rewritten as follows to show this revision as well as that recommended at the last meeting by Rask (*This Journal*, 25, 82):

#### DETERMINATION

Grind separately not over 25.5 g of malt as directed under 45. Collect the finely ground malt in mash beaker, carefully brushing in the malt particles remaining in mill. Without delay, adjust weight of contents to 25 g ( $\pm 0.05$  g). Transfer quantitatively to container (capacity ca 1 liter) in which infusion is to be made. Add 500 ml of freshly distilled  $H_2O$  and close container. Let infusion stand 2.5 hours at  $20^\circ$  ( $\pm 0.2^\circ$ ) and agitate by rotation at 20 min. intervals. Take care that in agitation of malt suspension as small a quantity as possible of grist is left adhering to inner surface of flask above level of the  $H_2O$ . (Mixing by inverting the flask is specifically cautioned against; gentle whirling of contents without splashing on sides of container has been found to be sufficient.) Filter infusion by transferring entire charge to 30–32 cm fluted filter (CS and S No. 588 or its equal in quality) contained in 175 mm funnel. Return first 50 ml of filtrate to the filter. Collect filtrate until 3 hours shall have elapsed from time the  $H_2O$  and ground malt were first mixed. Prevent evaporation during filtration period as far as possible by placing a watch-glass over funnel and some suitable cover around stem of funnel, resting on neck of receiver.

Immediately dilute 90 ml of the above fusion to 100 ml at  $20^\circ$ , transfer 10 ml of this fusion to a 250 ml volumetric flask, and bring to  $20^\circ$ . Add 200 ml of buffered stock soln from a fast-flowing pipet, all at  $20^\circ$ . Mix soln by rotating flask during the addition. Maintain the “starch infusion” mixture at  $20^\circ$  ( $\pm 0.2^\circ$ ) for exactly 30 min., timed on a stop-watch from time addition of starch was begun. Add 20 ml of 0.5  $N$  NaOH rapidly and mix well by whirling the flask. Make to mark at  $20^\circ$  and mix thoroughly. (For heating soln an electric plate is preferable to a gas flame.)

Determine reducing power by (a) Fehling’s modification, or (b) the ferricyanide modification.

(a) *Fehling’s modification.*—Boil 10 ml of the Fehling soln and 10 ml of  $H_2O$  in small flask with narrow neck (200 ml Erlenmeyer). Add from buret ca  $\frac{1}{2}$  of amount of above digested starch soln probably required and boil 15–20 seconds, rotating constantly. Remove from flame. If still decidedly blue, add more soln, boil ca 10 seconds, and again observe color. When blue color has been almost discharged, and after boiling gently ca 2 min., add 3 drops of a 1% aqueous methylene blue soln. Continue boiling and add more soln until 0.1 ml, or even 1 drop, upon boiling, discharges the blue color. (Color becomes violet-lavender as end point nears.)

Repeat titration, adding at once almost whole amount of digested starch required and proceed to end point as directed. Let amount of digested starch soln required to reach end point in this second titration be called A. Interrupt boiling as little as possible after indicator has been added, so that flask remains filled with steam, preventing much access of air. Upon cooling the blue color usually returns.

(b) *Ferricyanide modification*.—Proceed as directed in 47 and 48, p. 225, using a 5 ml aliquot (no soluble starch indicator is necessary). Subtract number of ml of 0.05 *N* Na thiosulfate used in titration from number of ml obtained in titrating the blank and multiply by 23 to obtain degrees Lintner on "as is" basis. Convert to dry basis in usual manner.

Degrees Lintner  $\times 4$  = mg of maltose equivalent. Report diastatic power values both as degrees Lintner and mg maltose equivalent until such time as degrees Lintner can be dropped.

(4) The methods for the sampling of hops and for the determination of leaves, stems, seeds, moisture, and total soft (alpha and beta) and hard (gamma) resins in hops, published in *This Journal*, 25, 292–296, and revised by the associate referee this year, were adopted as tentative. The changes are as follows:

(a) In 2(f), p. 293, 2nd paragraph, 2nd line, change the phrase "dropping the same" to read, "using a circulatory motion, and drop onto." In the same paragraph 2nd line from end after "0.01 gram" insert the following sentence: "By means of forceps remove all particles of spindles from the seed in the tared dish before weighing."

(b) In 3, p. 294, line 3, change the sentence to read, "Pass the hops evenly and slowly through the grinder, taking care to avoid choking of the orifice, thus preventing undue heating of the hops."

(c) In 4(3), p. 294, change "1 hour" to "2 hours," and in the 2nd line, 2nd paragraph, after the words "70 mm dish" add the following sentence: "The amount of hops and the dimensions of the dishes used are important for accurate results."

(d) In 6, line 4, before "100 ml" add word "about"; on p. 295, line 6, after the words "Wash the paper and funnel," add "thoroughly until all traces of resin are dissolved, and"; in next paragraph, line 4, after word "flask" add "or Soxhlet extraction flask," and change the remainder of this paragraph to read as follows: "Evaporate off the solvent by distillation on a water or steam bath at 60°C., using vacuum and driving off the last 5–10 ml by applying suction to the flask while it is kept immersed in the water or steam bath until constant weight is obtained (usually after 5 min.). Weigh the flask after it has stood near the balance for 30 min."; and change the last paragraph to read as follows: "Use vacuum during distillation and concentration of the petroleum benzine extract and drying of the residue. In place of either a distillation flask or Soxhlet extraction flask, a large sized aluminum moisture dish may be used. % of soft resin = weight of resin  $\times 100$ ."

(e) In 7(a), p. 295, par. 1, line 3, after word "flask," insert the words "or other suitable flask"; in line 4, after "60" insert the words "using vacuum and" and delete "CO<sub>2</sub> and"; in line 6 after the word "flask" insert the words "by warming"; at the end of par 1, on p. 296, change the statement in parentheses to read, "This quantity is usually sufficient to precipitate alpha resin from hops having average composition of 16–19% of soft resins"; and change the first sentence of the last paragraph to read as follows: "In determining the alpha resin content of hops with an exceptionally high or exceptionally low content of soft resins it will require 1 or 2 ml more or less of the lead acetate reagent for satisfactory results."



(5) The following methods for the determination of moisture, extract, fermentable extract, and protein in brewing sugars and sirups (*Am. Soc. Brew. Chem.*, 1940, pp. 2-8) were adopted as tentative:

## BREWING SUGARS AND SIRUPS

### 1 MOISTURE

Calculate by difference, as follows:

$$100 - \text{Extract \% (Plato)} = \% \text{ Moisture.}$$

### 2 EXTRACT

#### DETERMINATION

Accurately weigh ca 50 g of a well mixed, representative sample, dissolve in warm H<sub>2</sub>O, transfer quantitatively to a 500 ml flask, and bring volume up to 500 ml at 20°. After thorough mixing, accurately determine by means of a suitable pycnometer, the sp. gr. of this "10% soln" at 20°/20°. Standardize pycnometers and determine sp. gr. as directed under 42-44, pp. 157-160.

### 3 CALCULATION

Obtain the extract corresponding to the sp. gr. from the tables based upon determinations by Plato and published by the American Society of Brewing Chemists.

Calculate % extract in sample after correcting for sp. gr. of the "10% soln" as follows:

Extract % (Plato) =  $P \times B \times 500 / W$ , where P = extract % (Plato) of "10% soln"; B = specific gravity of "10% soln"; and W = actual weight (ca 50 g) taken.

Report to first decimal place.

*Degrees Baumé.*—Report equivalent Baumé degrees (Modulus 145) determined by reference to the tables published by the American Society of Brewing Chemists.

Report to first decimal place.

### 4 FERMENTABLE EXTRACT

#### DETERMINATION

Ferment 250 ml of the "10% soln" of the sample (2), with the equivalent of 5 g of washed, active brewers' compressed yeast at a temp. of 15-25°C. (60-77°F.) for 48 hours or until fermentation is complete. In the case of refined sugars and sirups, such as corn sirup, add to the soln before fermenting 0.8 g of secondary K<sub>2</sub>HPO<sub>4</sub> crystals, 1 g of primary NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, and 0.5 g of dried yeast extract, standardized for bacteriological culture media purposes, as nutrients. If such nutrient material needs to be added, redetermine the extract of the "10% soln" after the addition of the nutrient material, but before adding the yeast. Use fermentation flasks equipped with water seals and shake flasks several times a day during fermentation.

### 5 REAL EXTRACT

#### DETERMINATION

When fermentation is complete, filter the soln and determine real extract in the filtrate as directed under 4.

### 6 CALCULATION

Use the following formulas:

$$\text{Fermentable Extract (dry basis)} = \frac{(p - n)}{P} \times 100, \text{ and}$$

$$\text{Fermentable Extract (as received)} = \frac{(p - n)}{D} \times \text{extract in sample,}$$

where P = real extract of "10% soln" before the addition of any nutrients;  
 p = real extract of "10% soln" before fermentation; and  
 n = real extract of "10% soln" after fermentation.

If nutrients have not been used (as in case of malt sirups)  $p = P$ , and will have been determined under 2, and no re-determination before fermentation is required in such a case.

Report to first decimal place.

7

#### PROTEIN

Transfer 25 ml of the "10% soln" (2) to a Kjeldahl digestion flask, and proceed as directed under 18, p. 152.

Percent protein ( $N \times 6.25$ ) in original, "as is"

$$\text{sample} = \frac{(\text{ml } 0.1 \text{ N H}_2\text{SO}_4 - \text{ml } 0.1 \text{ N NaOH}) \times 0.0014 \times 6.25 \times 500}{25 \times W} \times 100,$$

where W = actual weight of sample used in preparing the "10% soln."

Report to second decimal place.

(6) The method for the determination of chlorides in beer (26, 155) was made official (final action).

(7) The method for the determination of sulfur dioxide in beer (21, p. 154) was made official (final action).

(8) The method for the determination of color in beer (2, p. 150) was made official (final action).

#### XV. WINES

The tentative method for the determination of sulfurous acid (38, p. 170), modified as shown in *This Journal*, 25, 82, was adopted as official (final action).

#### XVI. DISTILLED LIQUORS

No additions, deletions, or other changes.

#### XVII. BAKING POWDERS AND BAKING CHEMICALS

No additions, deletions, or other changes.

#### XVIII. COFFEE AND TEA

No additions, deletions, or other changes.

#### XIX. CACAO BEAN AND ITS PRODUCTS

(1) The method for the determination of milk protein in milk chocolate (official, first action), (*This Journal*, 25, 83) was made official (final action).

(2) The tentative method for the determination of milk proteins (9, 201) was deleted.

(3) The tentative method of calculating milk fat in milk chocolate (14, p. 204) was deleted.

#### XX. CEREAL FOODS

(1) The following method for the determination of iron in flour and bread was adopted as tentative:

## IRON IN FLOUR AND BREAD

## PREPARATION OF SAMPLE

Slice bread, allow to air-dry until in equilibrium with air, and crush to ca 20-mesh size on wooden surface with wooden rolling pin. (Grinding may be done in mill if experiments show no increase in Fe due to grinding on the particular material under examination. In general, grinding in mills increases the iron content.)

## REAGENTS

(a) *Ortho-phenanthroline soln.*—Dissolve 0.1 g of orthophenanthroline in ca 80 ml of  $H_2O$  at  $80^\circ$ , and after cooling, dilute to 100 ml.

(b) *Alpha-alpha dipyridyl soln.*—Dissolve 0.1 g of alpha-alpha dipyridyl (Eastman Co.) in  $H_2O$  and dilute to 100 ml.

(Keep Reagents (a) and (b) in cool, dark place and they will remain stable for several weeks.)

(c) *Hydroxylamine hydrochloride soln.*—Dissolve 10 g of hydroxylamine hydrochloride in water and dilute to 100 ml.

(d) *Magnesium nitrate soln.*—Dissolve 50 g of  $Mg(NO_3)_2 \cdot 6H_2O$  in  $H_2O$  and dilute to 100 ml.

(e) *Acetate buffer soln.*—Dissolve 8.3 g of C.P. anhydrous Na acetate (previously dried at  $100^\circ$ ) in  $H_2O$ , add 12 ml of glacial acetic acid, and dilute to 100 ml. (It may be necessary to redistil the C.P. glacial acetic acid and purify the Na acetate by recrystallization from  $H_2O$ , depending on amount of iron present.)

## PREPARATION OF REFERENCE CURVE

(1) Dissolve 0.1 g of analytical grade iron wire in 20 ml of HCl and 50 ml of water, and dilute to 1 liter. Dilute 100 ml of this soln to 1 liter. Each ml has 0.01 mg of iron (Fe). Or—

(2) Dissolve 3.512 g of ferrous  $(NH_4)_2SO_4$  in  $H_2O$  [ $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ ], add 2 drops of HCl, and dilute to 500 ml. Dilute 10 ml of this soln to 1 liter. Each ml has 0.01 mg of iron (Fe).

Make 10 solns with 2.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, and 45.0 ml, respectively, from the finally diluted stock soln. Also run a blank. Add 2.0 ml of HCl and dilute to 100 ml. Use 10 ml of each of these solns and follow the procedure as given under "Determination," beginning "add 1 ml. of hydroxylamine soln." Plot concentration against scale reading.

## DETERMINATION

Ash 10.0 g of flour or air-dry bread in platinum, silica, or porcelain dish (ca 60 mm diameter, 35 ml capacity) in accordance with method for ash, 5, p. 212. (Porcelain evaporating dishes of ca 25 ml capacity are satisfactory. Do not use flat-bottomed dishes of greater diameter than 60 mm.) Cool, and weigh if % ash is desired. Continue ashing until practically carbon free. To diminish ashing time, or for samples that do not burn practically carbon free, use one of the following ash aids: Moisten the ash with 0.5–1.0 ml of the  $Mg(NO_3)_2$  soln or with distilled  $HNO_3$ . Dry contents and carefully ignite in muffle to prevent spattering. (A white ash with no carbon results in most cases.) Do not add these ash aids to self-rising flour or bread (products containing salt) in a platinum dish because of vigorous action on the dish. Cool, add 5 ml of HCl, evaporate to dryness on the steam bath, dissolve residue by adding, accurately measured, 2.0 ml of HCl, heat for 5 min. on steam bath with watch-glass on dish, wash off the watch-glass with  $H_2O$ , filter into a 100 ml volumetric flask, cool, and dilute to volume. Pipet 10 ml of aliquot into a 25 ml volumetric flask, and add 1 ml of the hydroxylamine soln; in a few minutes add 5 ml of the buf-

fer soln and 1 ml of the ortho-phenanthroline or 2 ml of the alpha-alpha dipyridyl soln and make to volume. Read the intensity of color in a 2 inch cell on the neutral wedge photometer, using the No. 51 filter (ca 510 wave length) or other suitable instrument of equivalent precision. From the reading, determine the concentration of iron from the equation of the line representing the standard points or by reference to standard curve for known iron concentration. Determine blank on the reagents and make correction. Calculate amount of iron in the flour or bread as mg per pound. Rinse all flasks, beakers, funnels, etc., with  $H_2O$  before use, and filter all reagents to remove suspended matter.

(2) The following method for the determination of calcium was adopted as tentative:

#### CALCIUM

Use 50 ml aliquot of soln from preceding method for iron, dilute to ca 200 ml, and proceed as directed in 19, p. 339, as far as the words "and allow mixture," 3rd line from end. Proceed as directed under 48, p. 366, beginning "Let stand overnight" except for titration with 0.05  $N$   $KMnO_4$ . Calculate Ca as mg/lb.

(3) The electrometric procedure for the determination of hydrogen-ion concentration published in *This Journal*, 26, 109, was adopted as tentative.

(4) The following Hopkins revision of the Mannich-Lenz procedure for the determination of starch (*Canadian J. Research*, 11, 751 (1934)) was adopted as tentative:

#### STARCH<sup>1</sup>

##### REAGENT

*Calcium chloride soln.*—Dissolve 2 parts of crystalline  $CaCl_2 \cdot 6H_2O$  in one part of  $H_2O$  and adjust to density of 1.30 at 20°. (This soln contains ca 33% of  $CaCl_2$ .) Make faintly pink to phenolphthalein by adding 0.1  $N$   $NaOH$ . (Anhydrous  $CaCl_2$  may be used, but it is usually alkaline and requires the addition of acid to bring it to correct pH.)

##### DETERMINATION

Grind sample finely (100-mesh if possible) and weigh 2.0–2.5 g into a 50 ml round-bottomed centrifuge tube with lip. Wash with ether to remove fat, then with 10 ml of ca 65% by weight aqueous alcohol ( $d_{20} 0.88$ ) and stir thoroughly with a glass rod. Centrifuge (1) and pour off the soln. Repeat the washing until 60 ml of wash liquid has been used, stirring each time with same rod.

Stir residue with 10 ml of  $H_2O$  and pour into a 200–250 ml Erlenmeyer flask. Complete transfer by washing out with a total of 60 ml of the  $CaCl_2$  soln containing 2 ml of 0.8% acetic acid. Transfer rod to flask and bring mixture to boiling quickly over wire gauze, stirring frequently. Boil briskly for 15–17 min., taking precautions to prevent burning and foaming (2). Rub the particles on sides of flask down with the rod from time to time.

Cool soln quickly in running  $H_2O$  and pour into a 100 ml volumetric flask, rinsing thoroughly with the  $CaCl_2$  soln from a wash-bottle with a medium jet. (In making up to mark, it is permissible to destroy the froth by adding one drop of alcohol.)

After thoroughly mixing the sample pour ca 10 ml of the soln onto a fluted filter (Whatman No. 42 or 44), wetting paper completely. Allow filtrate to run dry and discard. Resume filtration, using a dry receiver, and collect 40–50 ml (3).

<sup>1</sup> *This Journal*, 10, 108; 11, 484; 12, 390; 13, 447; 14, 112; 15, 583; 16, 505; 17, 400; 18, 570; 19, 621; 20, 547; 21, 360, 394; 22, 523; 23, 489; 24, 118.

Polarize the liquid in a 10 cm tube, taking 2 sets of 10 readings each. (The averages of the two sets should agree within 0.006°.) Calculate the starch content as follows:

$$\text{Percentage starch (4)} = \frac{100 \times A \times 100}{1 \times 200 \times S} - \frac{50 \times A}{S}, \text{ where } A \text{ is observed rotation}$$

and S is weight of sample.

**NOTES:**

(1) If no centrifuge is available wash samples on a filter paper, using a Pt cone and slight suction.

(2) An Argand burner with a thin asbestos gauze will diminish foaming.

(3) For filtering aids, Celite with Pyrex glass filters and Hirsch-type funnel with asbestos and suction are recommended.

(4) 200 is arbitrarily taken as specific rotation for all starches until a better figure is worked out for individual starches. If a 200 mm tube and a saccharimeter are used, and 2 g of sample is weighed out and the mixture is made up to 100 ml, each °V will be multiplied by 4.3225 to give % of starch.

(5) The methods for the determination of moisture and fat by acid hydrolysis in fig bars and raisin-filled crackers, published in *This Journal*, 26, 305), were adopted as tentative.

## XXI. COLORING MATTERS IN FOODS

No additions, deletions, or other changes.

## XXII. DAIRY PRODUCTS

(1) The tentative method for preparation of sample for analysis of frozen desserts containing insoluble particles (*This Journal*, 25, 85), was adopted as official (first action), and the word "particles" was changed to "ingredients."

(2) The first line of the double dilution method for correcting the volume of precipitate in the optical determination of lactose in milk (16, p. 271; *This Journal*, 25, 608) was changed to read "Weigh 65.8 g (2 N weight) of milk into each of two flasks graduated at 100 ml and 200 ml, respectively" (official, first action).

## XXIII. EGGS AND EGG PRODUCTS

(1) The method for the determination of cholesterol published in *This Journal*, 25, 87, was adopted as official (final action).

(2) The open Carius method for the determination of salt in egg products (*This Journal*, 26, 352) was adopted as an alternative official method (first action).

## XXIV. FISH AND OTHER MARINE PRODUCTS

No additions, deletions, or other changes.

## XXV. FLAVORING EXTRACTS

No additions, deletions, or other changes.

## XXVI. FRUITS AND FRUIT PRODUCTS

(1) The volumetric method for the determination of P<sub>2</sub>O<sub>5</sub> published in *This Journal*, 25, 441, was adopted as tentative.

(2) The colorimetric method for the determination of  $P_2O_5$  (39, 347) was adopted as official (final action). First action as official is recorded in *This Journal*, 24, 39-41.

(3) The official gravimetric method for the determination of  $P_2O_5$  (38, 347) was deleted (first action).

(4) The gravimetric chloroplatinate method for the determination of potassium published in *This Journal*, 26, 326, was adopted as official (first action).

(5) The gravimetric cobaltinitrite method for the determination of potassium published in *This Journal*, 26, 330, was adopted as official (first action).

(6) The volumetric chloroplatinate method published in *This Journal*, 25, 232; the short gravimetric chloroplatinate method (*Ibid.*, 26, 473; 25, 434); and the short volumetric method (*Ibid.*, 26, 324) for the determination of potassium were adopted as tentative.

## XXVII. GRAIN AND STOCK FEEDS

(1) The following method for sampling feeding stuffs was adopted as tentative:

### SAMPLING FEEDING STUFFS

Insert a sharp, closed-end sampler horizontally into the package. Take cores from not less than 10% of the bags present unless this process necessitates cores from more than 20 bags, in which case take core from 1 bag for each additional ton represented. If less than 100 bags, sample not less than 10 bags; if less than 10 bags, sample all bags. Thoroughly mix portions taken on clean oilcloth or paper, reduce by quartering to quantity of sample required, and place in air-tight container.

(2) For the temperature of ashing, 600°C. was adopted as official (final action).

(3) The method for the determination of starch (34, p. 361) in condensed or dried milk products was made official (final action).

(4) The method for the determination of soluble chlorine published in *This Journal*, 26, 89, was adopted as official (first action).

(5) The following method for the determination of urea and ammoniacal nitrogen (*This Journal*, 24, 79; as revised, *Ibid.*, 25, 93) was adopted as official (final action).

## UREA AND AMMONIACAL NITROGEN

### REAGENTS

(a) *Standard acid*.—See II, 19(a) or (b).

(b) *Standard alkali*.—See II, 19(c).

(c) *Indicator*.—See II, 19(h) or (i).

(d) *Defoaming soln*.—Dissolve 50 g of diglycol stearate in 375 ml of benzol, 75 ml of ethyl alcohol, and 250 ml of dibutyl phthalate, with warming if necessary.

(e) *Urease soln*.—Prepare fresh soln by dissolving standardized urease in  $H_2O$  so that each 10 ml of neutralized soln will convert the nitrogen of at least 0.1 g of pure urea. *Suggested standardization procedure*.—To determine alkalinity of commercial urease preparation dissolve 0.1 g in 50 ml of  $H_2O$  and titrate with 0.1 N HCl,

using methyl red indicator. Add this quantity of 0.1 *N* HCl to each 0.1 g of urease in preparing the urease soln. To determine the enzyme activity prepare ca 50 ml of a neutralized 1% soln. Add different quantities of soln to 0.1 g samples of pure urea and follow with the enzyme digestion and distillation as directed in the determination. Calculate activity of the urease preparation from the quantity of this urease soln that converted the urea, thereby permitting complete recovery of the nitrogen by distillation.

(f) *Calcium chloride soln.*—Dissolve 25 g of CaCl<sub>2</sub> in 100 ml of H<sub>2</sub>O.

#### DETERMINATION

Place 2 g of sample in a Kjeldahl flask with ca 250 ml of H<sub>2</sub>O. Add 10 ml of the urease soln, stopper tightly, and let stand at room temp. 1 hour or at 40° for 20 min. Cool at room temp. if necessary. Use more urease soln if feed contains more than 5% urea (ca 12% protein equivalent). Rinse stopper and neck with a few ml of H<sub>2</sub>O. Add 2 g or more of MgO (heavy type), 1 ml of the CaCl<sub>2</sub> soln, and 5 ml of the defoamer soln, and connect flask with condenser by means of Kjeldahl connecting bulb. Distil 100 ml of the liquid into a measured quantity of the standard acid, and titrate with the standard alkali, using cochineal or methyl red indicator.

### XXVIII. MEAT AND MEAT PRODUCTS

The tentative method for the detection of agar agar (*This Journal*, 25, 93) was adopted as official (first action).

### XXIX. METALS IN FOODS

(1) The following method for the determination of fluorine and a rapid method restricted to fluorine residues on apples and pears, which is included in the same copy, were adopted as tentative:

#### FLUORINE\*

##### 1

#### PRINCIPLES

The general method specifies an ashing treatment of the sample with lime as a F fixative, isolation of F by means of a Willard-Winter distillation<sup>1</sup> from HClO<sub>4</sub>, and estimation in the distillate by a Th(NO<sub>3</sub>)<sub>4</sub> "back-titration" procedure. The technique and reagent strengths are designed to handle conveniently not more than 10.0 mg of F. Modifications of this general procedure, applicable to specific products, are described. A special procedure for the rapid determination of F as spray residue is also given.

##### 2

#### PRECAUTIONS AND INTERFERENCES

The analyst should control, by means of careful choice and purification of reagents, the magnitude of a determination blank. Details of purification of several of these reagents are given in 4. With care the blank will be low (1–3 micrograms of F). A large part of it will be a "distillation blank" apparently resulting from F leached from the glassware of the still during the distillation. This blank can be minimized by the preliminary treatment of the still (8), and it should be possible to deduce and correct for an average distillation blank if stills of the same material and design are routinely used; otherwise, each still must bear its special blank. New, unused stills will usually be found to exhibit a high blank, which will diminish to a constant low figure after several runs. Ashing utensils should be checked by blank runs with the fixative soln to ascertain whether or not they contribute appreciable F. Even Pt

\* The report of the Associate Referee on Fluorine, of which these methods are a part, will appear in a later number of Vol. 27 of *This Journal*.

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 5, 7 (1933).

vessels may become contaminated (owing presumably to a slight calcium content) if they have been used recently for HF volatilization of silica. In addition, such blank runs are useful for the testing not only of the reagents and apparatus used in the method but also the evaporators, hoods, muffles, and laboratory atmosphere for the presence of fluoride fumes and dust. HF bottles, if permitted in the same laboratory, should be sealed immediately after use, and contamination from roach powders should be avoided. Ordinary tap  $H_2O$  may be a source of F contamination, since 1 ml of a water containing 2 p.p.m. of F will contribute 2 micrograms of F if allowed to remain or to dry in a still. For this reason all glassware (still, flasks, burets, etc.) should be routinely rinsed with distilled water, preferably redistilled from alkaline permanganate. Filter papers may contribute small (microgram) quantities of F, and glass filters are preferred should a filtration be required in the microdetermination.

*Interferences* are gelatinous silica, aluminum, and boron compounds, which repress the evolution of F as  $H_2SiF_6$  in the distillation; materials such as nitrates, nitrites, peroxides, Cl,  $SO_2$ , and  $H_2S$ , which act upon the indicator in the titration or otherwise interfere; halides (chloride), which distil to give excessive acidity in the distillate; and phosphates and sulfates, which react with Th in the titration to give high results. The procedure is so designed that most of these interferences are automatically eliminated, but the analyst should be on guard against their possible occurrence under unusual circumstances.

### METHOD (GENERAL)

#### 3

#### APPARATUS

(a) *Fluorine still*.—A Claissen-type distilling flask of 100–125 ml size is most practical for general work. It must be of Pyrex glass, and the auxiliary neck should be sealed off immediately above the side-arm to prevent pocketing and refluxing of distillate. The still should be as small and simply designed as practicable, in fact ordinary distilling flasks can be used for some work and they are slightly more efficient than the Claissen type, except that there is more danger of spraying over of distilling acid. The still is equipped with a dropping funnel and 0–150° thermometer, the latter extending to within  $\frac{1}{4}$ " of bottom of flask, so that the bulb is immersed in the boiling acid mixture. Acid-alkali washed beads, preferably of Pyrex, should be on hand. Rubber stoppers should be previously cleaned by boiling in 10% NaOH. All-glass apparatus with interchangeable accessories is convenient, especially in routine work, and eliminates the need for rubber stoppers. While not entirely necessary for heating the still, the use of a Wood's metal bath, adequately shielded, will prevent undue decomposition of  $HClO_4$ , and aid materially in securing a low-acid distillate. If the metal bath is used, care should be taken that the flask is not immersed so deeply that the bath level is above that of the liquid in the flask; if the bath is not used, transite or asbestos shielding boards are essential, and the flask should be heated through a small hole in such a shield by a low "clean" flame. At the analyst's option, distilling  $H_2O$  may be added as steam instead of through the dropping funnel, and an electric boiler<sup>2</sup> is a convenient steam generator. If steam is used, the inlet tube should dip below the surface of the liquid in the still. One advantage in adding the distilling  $H_2O$  through a funnel is that the last portions of rinse  $H_2O$  used in transferring an ash can be used in the distillation. If the funnel plug is thinly notched with a sharp file on either side of the bore, dropping rate can be more easily controlled, and the end of the funnel stem need not extend into the liquid in the still. The still is used in conjunction with a clean straight-tube condenser, no longer than necessary for adequate cooling. (A vertical arrangement of the condenser will conserve bench space.)

<sup>2</sup> *This Journal*, 21, 684 (1938).



(b) *Nessler tubes*.—Tall form, 50 and 100 ml sizes, glass-stoppered type preferred. Matched in sets of at least six. (The 100 ml size will be used more frequently in the general procedure.)

(c) *Additional apparatus*.—In addition there will be required (2) carefully cleaned and tested Pt, or well-glazed porcelain dishes of at least 100 ml size; 150 ml volumetric flasks, or if these are not available, the 200 ml size; and 10 ml burets (conveniently automatic) to deliver the various solns required in distillation and titration. An overhead radiant heater will be found invaluable for the drying and preliminary charring of samples, especially those of the high sugar type.

## 4

## REAGENTS

(a) *Lime suspension*.—Carefully slake ca 56 g (1 mol) of low F lime (ca 2 p.p.m. of F) with ca 250 ml of H<sub>2</sub>O and add 250 ml of 60% HClO<sub>4</sub> *slowly* and with stirring. Add a few glass beads and boil down to copious fumes of acid; then cool, add 200 ml of H<sub>2</sub>O, and boil down again. Repeat the dilution and boiling down once more; cool, dilute considerably, and filter through a fritted-glass filter if a precipitate of silica has appeared. Pour the clear soln, with stirring, into 1 liter of 10% W/V NaOH, allow precipitate to settle, and siphon off supernatant liquid. Remove Na salts from precipitate by washing 5 times in large centrifuge bottles, shaking up substrate thoroughly each time. Finally, shake precipitate into suspension and make to 2 liters. Preserve in paraffined bottles. (100 ml of this suspension should give no appreciable F blank when evaporated, distilled, and carried through the titration procedure described below.) Always shake suspension up well before using.

(b) *Perchloric acid soln*.—60%. Dilute C.P. HClO<sub>4</sub> with 3–4 volumes of H<sub>2</sub>O and boil down to original volume. Repeat, and preserve in Pyrex.

(c) *Sulfuric acid soln*.—Carefully mix equal volumes of C.P. H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O, boil down to fumes, cool, dilute *carefully*, boil down once more, and dilute to 1+1 volume.

(d) *Silver perchlorate soln*.—50% W/V.

(e) *p-Nitrophenol indicator*.—0.5% W/V alcoholic soln.

(f) *Potassium hydroxide soln*.—Exactly 0.05 N.

(g) *Potassium chloride soln*.—0.05 N, 3.727 g/liter.

(h) *Hydroxylamine hydrochloride soln*.—1.0% W/V.

(i) *Hydrochloric acid*.—Exactly 0.05 N.

(j) *Alizarin indicator*.—0.01 % soln of sodium alizarin sulfonate (Alizarin Red S).

(k) *Thorium nitrate soln*.—0.25 g of Th(NO<sub>3</sub>)<sub>4</sub>·12H<sub>2</sub>O or 0.20 g of Th(NO<sub>3</sub>)<sub>4</sub>·4H<sub>2</sub>O per liter.

(1) *Potassium silicofluoride*.—If pure K<sub>2</sub>SiF<sub>6</sub> is not obtainable, prepare as follows: Introduce, through a dropping funnel, a saturated soln of NaF, or a suspension of crude K<sub>2</sub>SiF<sub>6</sub>, into a 500 ml Claissen distilling apparatus containing 60 ml of 1+1 H<sub>2</sub>SO<sub>4</sub>, some glass beads, and 10–20 g of powdered silica (or glass) maintained at a boiling temp. of 120–125°. Conduct distillate into a soln of ca 25 g of pure KCl in H<sub>2</sub>O, held at a simmering temp. on a hot plate so that distillate volumes do not become excessive. If necessary, add additional H<sub>2</sub>O to the mixture by means of a dropping funnel placed in the side-neck of the still. Regulate the rate of addition of fluoride to the still and the temp. of the condensing H<sub>2</sub>O so that the side-arm and condenser do not become clogged with the evolved H<sub>2</sub>SiF<sub>6</sub>, which tends to lodge as a gelatinous mass. K<sub>2</sub>SiF<sub>6</sub> is formed in the receiver and although entirely crystalline it assumes the appearance of a gelatinous substrate owing to the small difference between its refractive index and that of the aqueous soln. When a substantial amount has been collected, pour contents of receiver into large centrifuge bottle and wash repeatedly by means of the centrifuge (shaking up precipitate thoroughly each time), until the washings are chloride free by test. Collect on a

Büchner funnel and either allow to air-dry or bring to constant weight in vacuo at 50–70°. Determine purity by means of a Travers titration<sup>3</sup> at boiling temp. with 0.2 N NaOH (1 ml = .01101 g of  $K_2SiF_6$ ); also by conversion to  $K_2SO_4$  by treating 0.3–0.4 g in a deep Pt dish with a little  $H_2O$ , then  $H_2SO_4$ , plus a little HF, fuming off the excess acid carefully (if overheated, the mixture has a great tendency to spatter), and heating to constant weight of  $K_2SO_4$  at 650°. With glass apparatus an entirely pure product is not usually obtained as some contamination with silica results from the leaching effect of the vapors upon the condenser. A pure product can be obtained by use of a Pt still. 0.9660 g of pure  $K_2SiF_6$  made to 1 liter gives a stock soln containing 0.5 mg of F/ml. Much more will not dissolve. Prepare such a soln, correcting this weight of 0.9660 by the purity factor of the  $K_2SiF_6$  (the figure for purity obtained from the average of the two above methods of assay). Preserved in a paraffined bottle, the soln will keep indefinitely.

Prepare the soln *used in the titration* (9) by diluting 20 ml of this stock soln to 1 liter (1 ml = 10 micrograms of F). It will retain its strength for several weeks in ordinary volumetric ware.

(m) Check the titer of the Th soln against the standard (10 microgram/ml) fluoride soln as follows: Measure 10, 20, 30, etc., up to 80 micrograms of F into 100 ml Nessler tubes, and add 4.00 ml of the 0.05 N HCl (2.00 ml if 50 ml Nessler tubes are used, and carrying the range to only 50 micrograms of F for this size tube).<sup>4</sup> Dilute mixture to ca the 80 (or 40) ml mark and add 1.00 ml of the 1.0%  $NH_4OH \cdot HCl$  soln. Mix, then add exactly 2.00 ml of the alizarin indicator (or 1.00 ml for the smaller tube) and measure in the Th soln from a buret, mixing frequently until, when sighting down the tube towards a white reflecting surface, an incipient pink or salmon pink color is observed. Add a little  $H_2O$  from time to time so that the soln is nearly to mark as the end point is approached. Finally, make exactly to mark and mix thoroughly before checking the final end point. Do not shake the tubes violently (5–6 gentle inversions are sufficient). Make an effort to secure an end-point shade intermediate between the yellowish green of the acid indicator and the reddish purple of the fully developed Th lake. Carry through with the series and plot ml of Th soln against ml of standard fluoride to obtain a rough equivalence curve for the two solns. Depending upon the quantity of F known to be present, add the Th soln in 1–2 ml portions at first, with final additions of 0.25 ml.

## 5

### SAMPLE PREPARATION

Methods of sample preparation are designed to furnish a representative sample in a workable quantity of material and to get the sample in condition for the final distillation. Mineralization by an ashing procedure is usually involved. Some mineral food products can be dissolved in, and distilled from  $HClO_4$  (8) provided no interferences appear in the final distillate. In general, 10–20 g of dry material, 50–100 ml of liquid samples, and 50–100 g of undried food products or plant material can be taken for analysis, depending upon the expected F content, and the interferences, such as excessive chloride, which the use of large samples may introduce. If adequate grinding and mixing equipment is available, it is often feasible to prepare large quantities of material (vegetables, mixed foods) and to take aliquant portions for analysis.<sup>5</sup> Dry plant materials, feeds, bonemeal, etc., can be ground to convenient size in a Wiley mill and thoroughly mixed before the sample is taken. The following special methods for certain products are indicated.

**6 Direct ashing.**—(Applicable to fibrous (not highly fatty) food materials, liquid samples, and in general to all foods that can be thoroughly wet with an aqueous fixative soln. This procedure will apply to the majority of food products.)

<sup>3</sup> *This Journal*, 14, 253 (1931); *Methods of Analysis*, A.O.A.C., 1940, 20, 21, p. 50.

<sup>4</sup> *This Journal*, 24, 350 (1941).

<sup>5</sup> *Ind. Eng. Chem., Anal. Ed.*, 13, 93 (1941).

Weigh a suitable portion of the prepared sample into a clean Pt dish and add 25 ml of the lime suspension. (Porcelain casseroles or dishes are second choice because they may contribute small quantities of F, and alumina, to the sample.) Mix in the lime suspension with a glass rod, adding additional  $H_2O$  if necessary; rinse and remove rod. Dry *thoroughly* on steam bath or in hot air oven, then slowly char sample by heating over a low flame or electric stove with heat control. An overhead radiant heater is very convenient for both drying and charring the sample. Control excessive swelling of high sugar foods by playing a small flame over the sample surface from time to time and char these products *slowly* so that excessive acidity is not generated. When the sample is charred past danger of catching fire, place in muffle and ash at  $600^\circ$ . (For very small samples and minimum blanks it may be advisable to cover the ashing vessel with an inverted Pyrex petri dish while ashing.) When a clean ash is obtained, cool dish and wet ash with ca 10 ml of  $H_2O$ . (A small amount of unburned carbon does not interfere, but if much is apparent, dry down and repeat the ashing.) Cover dish with a watch-glass and cautiously introduce under the cover a little of the  $HClO_4$  in amount just sufficient to dissolve the ash. Rinse down cover with a little  $H_2O$  and transfer soln to the freshly prepared F still (8) through a long-stemmed funnel. Rinse dish with remainder of distilling acid, using ca 20 ml in all, and adding and transferring in several small portions. *Do not prolong the transferring operation.* Finally rinse funnel and stirring rod into dish, assemble still and complete rinsing of dish with several small portions of  $H_2O$ , pouring these into the dropping funnel of the still. If distilling  $H_2O$  is added as steam (3(a)) rinse dish with a little additional  $H_2O$  and add directly to acid mixture in still, but avoid excessive initial volume. Add ca 6 Pyrex beads and sufficient  $AgClO_4$  soln to precipitate all chloride. (Excess  $AgClO_4$  in reasonable quantity does no harm, and sufficient solid  $Ag_2SO_4$  may also be used.) Proceed as directed in 8.

**7 Preliminary distillation.**—(Necessary with certain products of high phosphate content, such as calcium phosphate and bone meal, in order to eliminate the distilled phosphoric acid that may be present in appreciable quantities in first distillates. Also advisable with certain fatty materials that may not be thoroughly wet with the lime fixative, thus causing F loss in a direct ashing procedure.)

(a) *For inorganic phosphatic materials*, such as calcium phosphate, weigh sample, usually 10 g, into still, add a few glass beads, sufficient  $AgClO_4$  to precipitate possible chloride, and ca 20 ml of the  $HClO_4$ . If the inorganic phosphatic material does not contain excessive Ca, use a similar quantity of the 1+1  $H_2SO_4$ . Distil at  $135\text{--}140^\circ$ , collecting ca 200 ml of distillate. (For this preliminary distillation, extreme care in the securing of a low-acid distillate is not essential.) Evaporate distillate to dryness in Pt after addition of an excess of the lime suspension, assuring alkaline conditions by testing with a drop of phenolphthalein indicator. (If  $H_2SO_4$  is used in this preliminary distillation add to distillate a few drops of *F-free* 30%  $H_2O_2$  to remove possible sulfites.) Heat dried residue at  $600^\circ$  for a few minutes to destroy indicator residues and possible  $Cl_2$ -containing compounds. Transfer contents of dish to a freshly prepared still (8) with the 20 ml of distilling  $HClO_4$  as directed in 6 and proceed with the final distillation as directed in 8.

Take 20 ml samples of sirupy phosphoric acid and collect at least 300 ml of first distillate at  $135^\circ$ , allowing the phosphoric acid to function as its own distilling acid. (More distillate is necessary because the phosphoric acid is less effective as a F distilling acid.) Neutralize with the lime suspension, evaporate to dryness, transfer to prepared still as directed above, and proceed as directed in 8.

(b) *For organic phosphatic materials*, such as bone meal, feed supplements, etc., give the sample a preliminary ashing treatment to destroy most of the organic matter. For this purpose, moisten sample with sufficient of the lime suspension, dry, char, and heat at  $600^\circ$  for 2–3 hours. Transfer ashed material to still, which contains

several beads and sufficient  $\text{AgClO}_4$  to precipitate chloride, with 20 ml of the distilling acid ( $\text{HClO}_4$  or  $\text{H}_2\text{SO}_4$ , depending upon the Ca content of the sample) as directed in 6, and continue as directed in 7(a) "Distill at 135–140°, etc."

*Certain organic phosphatic materials* (small samples of bone, 2–5 g, such as the entire bones of small test animals) in which the quantity of organic matter is not excessive, may be placed in the still and distilled directly as directed in 7(a) without a preliminary ashing. If the sample bears appreciable Ca (bone samples), use  $\text{HClO}_4$ , with reasonable precaution; if the organic phosphatic material does not contain excessive Ca, use 1+1  $\text{H}_2\text{SO}_4$ . In either case add more lime to first distillates and ash for a longer period of time in order completely to destroy distilled organic matter (fatty acids). Transfer contents of dish to freshly prepared still (8) with the 20 ml of  $\text{HClO}_4$  as directed in 6 and proceed with the final distillation (8).

*Baking powders* (calcium phosphate and combination types) are handled as follows: Place 10 g of sample in deep, covered Pt dish or casserole and slake cautiously with ca 20 ml of the lime suspension. After action subsides, rinse cover, dry contents of dish thoroughly, and ash at 600° for 2–3 hours. After completing the ashing step, cool dish and, because of excess of carbonate in the ash, treat it with several small portions of warm  $\text{H}_2\text{O}$ , breaking up with a flattened stirring rod, and transfer leachings to still. Then transfer remaining contents of dish with the 20 ml of distilling  $\text{HClO}_4$ , avoiding excessive effervescence when acid is added to carbonate soln in still. Add several glass beads and sufficient  $\text{AgClO}_4$ , and proceed as directed in 7(α) "Distil at 135–140°, etc. . . ." With combination or sodium aluminum sulfate baking powders, collect at least 400 ml of the preliminary distillate (7(d)).

(By the use of a specially trapped still it is possible to analyze highly phosphatic inorganic or thoroughly ashed materials, and phosphoric acids with a single distillation. The special trap, or scrubber, consists of 12–15 g of small, hollow glass beads supported in the side-neck of the 125 ml Claissen flask by several indentations punched in the side wall, and capped by a glass disk or the inverted bottom of a 15 mm test tube. After construction of the glass-bead scrubber the side-neck is sealed off immediately above the outlet tube. The beads in the scrubber are always wet with a little phosphoric acid before the distillation to furnish a liquid acid phase.) Take 20 ml of sirupy phosphoric acid, and 10 g samples of calcium phosphate with 20 ml of the  $\text{HClO}_4$  for the distillation, and collect at least 400 ml of distillate at 135°. With the single distillation observe the precautions outlined in 5, and also in 8 regarding the neutralization of final distillates. (Distillates should show practically negligible acidity. The presence of only traces of distilled phosphoric acid will vitiate the titration; as little as 20 micrograms of  $\text{P}_2\text{O}_5$  will definitely interfere. Accordingly, if the single distillation procedure is to be applied with confidence, it is necessary to test the distillates obtained from phosphatic materials by means of the special still for the presence of this interference.) For a convenient test utilizing Schricker's reagent,\* add 5 ml of a 1+9 dilution of this reagent to 45 ml of distillate in a 50 ml cylinder or Nessler tube, mix, and immerse in steam bath for 5–10 min. Compare against a blank by sighting down the tube. A blue or blue-green color denotes phosphate and as little as 5 micrograms (as  $\text{P}_2\text{O}_5$ ) is readily detected. If the distillate shows traces, make sure that such quantities are below an interference level of 20 micrograms before applying the titration to additional portions of the distillate. The test with Schricker's reagent is also useful in the usual double distillation procedure where a phosphate interference is possible. Use of the special trap will save time where highly phosphatic materials are handled as routine but it is not justified in ordinary work because of poor efficiency owing to excessive refluxing in the distillation.

\* This Journal, 22, 167 (1939).

(c) *Fatty and oily food materials* (oil-packed foods, oil meals, certain meats, etc., also the entire undried and unground organs of test animals), where there is danger of F loss through incomplete wetting with the lime fixative soln, may be handled as follows: Weigh an appropriate quantity of sample, usually 10 g, into still, and add silver (preferably 0.1–0.2 g of solid  $\text{Ag}_2\text{SO}_4$ ), several glass beads, and 20–25 ml of the 1+1  $\text{H}_2\text{SO}_4$ . Distil at 130–135° and collect 200–250 ml distillate. If foaming is excessive, increase quantity of distilling acid, and where necessary use a larger (250 ml) still. (The oil or fat of many of these products will tend to prevent foaming; and in some instances the use of a piece of purified paraffin about the size of a pea is an additional aid.) Oxidize distillate by cautious addition of 2–3 ml of *F*-free 30%  $\text{H}_2\text{O}_2$  to remove sulfites, evaporate in Pt with an excess of the lime suspension, and ash residue at 600° until clean. Proceed as directed in 7(a), beginning “transfer contents of dish to freshly prepared still, etc.” Handle pure oils by a similar procedure, and with these products use a 10 g sample with 25 ml of 1+1  $\text{H}_2\text{SO}_4$  and carry temp. at first to ca 170° to saponify; then carefully bring temp. down to 140° with the distilling  $\text{H}_2\text{O}$  and collect 250 ml of distillate. (It will probably be necessary to use a higher reading thermometer for this procedure.) Oxidize distillate with 30%  $\text{H}_2\text{O}_2$  and evaporate to dryness after adding an excess of the lime suspension. Ash at 600° and after a brief preliminary ash period remove dish, add a little  $\text{H}_2\text{O}$  plus an additional 1–2 ml of the peroxide to remove sulfides, dry, and complete ashing. Proceed as directed previously in this paragraph.

(d) *Aluminum and boron compounds* repress the evolution of F and complete isolation of their F content necessitates a preliminary distillation at elevated temperature. For this purpose, weigh sample, usually 5–10 g, into still, add 25 ml of the 1+1  $\text{H}_2\text{SO}_4$ , and conduct first distillation at 160–165° (special thermometer), collecting 300 ml of distillate. Oxidize distillate with 30%  $\text{H}_2\text{O}_2$  as above, evaporate down in Pt with an excess of lime suspension, incinerate briefly at 600°, and proceed as directed in 7(a), beginning, “Transfer contents of dish to freshly prepared still, etc.”

## 8

## FINAL DISTILLATION

The final distillation is always made from  $\text{HClO}_4$  and due precautions are taken to secure a low acid distillate (3(a)). Interferences, such as organic matter, phosphate, sulfate, etc., must be absent in the distillate, hence it must be made with careful temperature control in the presence of enough Ag salt to repress chloride evolution (2). It is well to check distillates for the presence of possible phosphate as directed in 7(b), and where advisable, as in 7(d), to test for sulfate with a little dilute  $\text{BaCl}_2$  soln. The  $\text{HClO}_4$  used in the final distillation is usually employed in transferring the ash to the still (6). A few acid-alkali washed beads are used to control bumping. The use of powdered silica does not appear necessary for the microdetermination.

In order to promote better recoveries, and to minimize and render constant the distillation blank discussed later in this paragraph, prepare the still by a special cleaning process before this transfer by treating it with hot 10%  $\text{NaOH}$  after each run, flushing out with tap  $\text{H}_2\text{O}$ , and then rinsing with distilled  $\text{H}_2\text{O}$ . Occasionally (at least once a day, and especially after it has stood idle for any length of time), treat the still additionally by boiling down 15–20 ml of the 1+1  $\text{H}_2\text{SO}_4$  until it is filled with fumes. Allow to cool and *thoroughly* rinse out.

At this stage the prepared sample has been transferred to the specially treated still, as directed above, for the final isolation of F. Begin the distillation, and when the temp. reaches 137° maintain at this point ( $\pm 2^\circ$ ) by adding  $\text{H}_2\text{O}$  from the dropping funnel (3(a)). Catch distillate in a 150 ml. volumetric flask, or, if one of these is not at hand, use a 200 ml flask and after a few ml of distillate has been col-

lected, add 1–2 drops of the *p*-nitrophenol indicator. Maintain the distillate alkaline to this indicator (faintest perceptible yellow) by adding a drop or two of the 0.05 *N* KOH from a 10 ml buret from time to time during the distillation, swirling the receiver contents. Regulate this addition of alkali so that the distillate is neutralized (within 1 drop of alkali) as it approaches the mark. Note carefully the volume of alkali used. Make the distillate to mark and mix thoroughly. Do not allow a F distillate to stand more than a few minutes before making neutral.

If the sample contains such large quantities of chloride that bumping in the still can not be controlled, dissolve the ash of another sample, and acidify slightly with  $\text{HClO}_4$ . Dilute considerably and precipitate chlorides in the dish with the  $\text{AgClO}_4$ , avoiding large excess. Filter through a glass filter, wash precipitate *thoroughly* with hot  $\text{H}_2\text{O}$ , and evaporate filtrate and washings to dryness after adding an excess (to alkalinity) of the lime suspension. Transfer residue to still with the  $\text{HClO}_4$  and repeat distillation as directed above.

## 9

## TITRATION

Place aliquot of final distillate in Nessler tube and mark "S" (sample). (Optimum quantities of F to be titrated are 60–70 micrograms for the 100 ml Nessler tubes and 30–40 micrograms for the 50 ml size, and it is well to make an exploratory titration on a small aliquot to check the approximate F content of the distillate.) Add the 0.05 *N* HCl, 4.00 ml for the 100 ml tubes and 2.00 ml for the 50 ml size, and 1.00 ml of the hydroxylamine soln. Dilute to ca 90 (or 40) ml, mix well, then add the proper amount of the alizarin indicator (2.00 or 1.00 ml) and mix in. Always add and mix in the hydroxylamine before adding the indicator. Prepare a blank tube ("B") by adding the proper amount of HCl and hydroxylamine, and a quantity of the 0.05 *N* KCl soln representing the same proportion of the total volume of 0.05 *N* KOH used to neutralize the distillate as the aliquot volume taken for the sample tube represents of the total distillate volume. (Thus, if 1.00 ml of 0.05 *N* KOH was used to neutralize a distillate volume of 150 ml and the aliquot taken for tube "S" was 75 ml, add 0.5 ml of the 0.05 *N* KCl to tube "B.") Dilute and mix, allowing slightly more head-space than in the sample tube. Then add the proper volume of alizarin indicator and mix.

Measure the Th soln into tube "S," mixing between additions, until an end-point of about the proper shade is reached. Dilute to mark, mix, and check this end-point shade. Note from the curve (4(m)) the approximate volume of standard fluoride soln corresponding to this volume of Th soln, and add ca 0.5 ml *less* than this quantity of standard fluoride to "B." Mix in, then add exactly the same volume of Th soln as was added to "S" duplicating approximately the increments in which it was added and the number of mixings. Dilute nearly to mark and compare colors of "S" and "B." (If the volume of standard fluoride added to "B" was properly chosen, this tube should be only slightly pinker in shade than the sample tube.) Bleach the "B" tube to an exact match with tube "S" by adding more of the standard fluoride to the former in increments of 1–2 drops, mixing gently between additions. Make to mark for the final comparison and observe the usual precautions of allowing bubbles to subside and of transposing tubes when final comparisons are made. (At the match-point, the fluoride content of tube "S" equals the quantity added to tube "B.") Check this end point by adding 1–2 drops in excess of standard fluoride to tube "B." A distinct over-bleach should develop.

Repeat the titration on aliquots of different size to obtain the total quantity of F distilled. If time is available, repeat the entire determination with a different weight of sample.

*For precise work*, evaluation of the reagent and of the distillation blank is necessary\* (2). Determine the distillation blank by making several distillations with the

prescribed quantities of  $\text{HClO}_4$  and  $\text{AgClO}_4$  from the freshly cleaned still, titrating the distillate as directed above with as large an aliquot as practicable. The average of the values found should not be greater than 2–3 micrograms of F. If quantities found by individual blank runs are too small to be determined accurately, make five or more separate distillations and evaporate the distillates, 150 ml each time, successively in the same Pt dish for a final distillation and average blank figure. The distillation and total determination blanks can usually be combined by carrying a run (with the same quantities of reagents and similar evaporation and ashing treatment) through the entire procedure. The reagents and manipulations should increase the distillation blank but little.

Calculate total quantity of F distilled from quantity found in aliquot titrated, subtract proper blank, and refer net figure to weight of sample taken. If the double distillation procedure was used, make appropriate correction.

## RAPID METHOD RESTRICTED TO FLUORIDE RESIDUES ON APPLES AND PEARS

### 10

#### PRINCIPLES

This method utilizes the acid filtrate from the strip soln of apples and pears when prepared with *HCl rinse and acidification*.<sup>7</sup> An aliquot of filtrate is oxidized colorless with  $\text{KMnO}_4$ , the solution is then reduced with hydroxylamine, and a back titration is conducted upon a sub-aliquot in Nessler tubes;  $\text{Zr}(\text{NO}_3)_4$  is used in the titration, with purpurin (1, 2, 4, trihydroxyanthraquinone) as indicator. The principle of the back-titration, as applied here, is similar to that employed in the general method where  $\text{Th}(\text{NO}_3)_4$  and alizarin occupy similar roles. The method is based, in general, on the work of Kolthoff and Stansby.<sup>8</sup> Provision is made for the renewal of interfering anions, and the high acidity employed in the titration minimizes interference of metals that would otherwise lake with the indicator.

### 11

#### APPARATUS

- (a) *Volumetric flasks*.—50 ml.
- (b) *Nessler tubes*—50 ml glass-stoppered, tall form, matched for height and color (14).

### 12

#### REAGENTS

- (a) *Mixed nitrate soln*.—Dissolve 3.0 g of  $\text{Ba}(\text{NO}_3)_2$  and 2.0 g of  $\text{Th}(\text{NO}_3)_4 \cdot 4 \text{H}_2\text{O}$  in  $\text{H}_2\text{O}$  and make to 100 ml.
- (b) *Potassium permanganate soln*.—Saturated; ca 6% W/V.
- (c) *Hydroxylamine hydrochloride soln*.—5% W/V.
- (d) *Ferrous chloride soln*.—Dissolve ca 1.0 g of Fe powder or wire in 50 ml of 1+1 HCl, dilute, and filter into a 500 ml volumetric flask. Add a few ml of the hydroxylamine hydrochloride soln and make to mark. Dilute still further, before use, if desired.
- (e) *Purpurin indicator*.—0.01% W/V in alcohol. Dissolve 25 mg of the pure dye in 95% alcohol, heating if necessary, and make to 250 ml with the same solvent. Prepare fresh weekly.
- (f) *Zirconium nitrate soln*.—Dissolve 1.00 g of  $\text{Zr}(\text{NO}_3)_4 \cdot 5 \text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , acidifying with 20 ml of HCl, and dilute to 1 liter. Filter if not clear.
- (g) *Standard fluoride soln*.—So dilute a stock soln of pure NaF that 1 ml = 36.4 micrograms of F.

<sup>7</sup> *Methods of Analysis*, A.O.A.C., 1940, 30, p. 407.

<sup>8</sup> *Ind. Eng. Chem., Anal. Ed.*, 6, 118 (1934).

## 13

## PROCEDURE

Place 20 ml of the well-mixed acid strip filtrate (10) in 50 ml volumetric flask. Add 2.0 ml of the mixed nitrate soln, then 4.0 ml of the  $\text{KMnO}_4$  soln. Rinse down neck of flask with a little  $\text{H}_2\text{O}$  and place on an active steam bath for 5 min. Remove flask, and while still hot, add the hydroxylamine hydrochloride soln from a buret, slowly and with swirling, until the  $\text{MnO}_2$  is discharged and soln is colorless. Add ca 0.5 ml of this reagent in excess. (Appreciable phosphate is revealed as flocculent  $\text{Th}_3(\text{PO}_4)_4$ , and sulfate as the precipitate with barium. Sometimes permanganate is occluded in this sulfate and/or phosphate precipitate and a pink color tends to persist but does not interfere.) Cool, make to mark, and filter. (Filtrate must be clear. If there is perceptible turbidity, return filtrate through filter several times if necessary, until filtrate is *brilliant*.) Pipet 25 ml of clear filtrate into Nessler tube and mark "S."

For a blank or comparison tube use 25 ml of "blank" soln containing the reagents as used in the method and prepared as follows:

Make 50 ml of the 10% sodium oleate soln (13, p. 397, *Methods of Analysis*, A.O.A.C., 1940), 50 ml of 30% W/V NaOH soln and 15 ml of HCl to 1 liter. Acidify portions with a one-tenth volume of HCl as if the soln were an actual "strip," and filter, returning filtrate thru filter until perfectly clear. (Chilling the soln and shaking vigorously will "churn" the precipitated oleic acid and aid in obtaining a clear filtrate.) Carry 20 ml portions of the acidified filtrate thru the procedure exactly as directed above. (It is best, in order to duplicate more closely the conditions of the actual determination, to use the 50 ml volumetric flasks and 20 ml aliquots in preference to working up larger aliquots with correspondingly larger amounts of reagents. After being made to volume and filtered, the blank solns may be combined to form a supply of "blank." Ten portions worked up as above yield ca 500 ml of blank, or enough for almost 20 determinations.)

Add 25 ml of this "blank" to a second Nessler (Tube "B"), and to both tubes "S" and "B," add 15.0 ml of HCl measured as carefully as possible from a graduate. (Always add the acid to the soln instead of vice versa.) Mix, and match the tubes for color. The "S" tube will usually be found to have a slight greenish tint in comparison with the "B" tube, due presumably to traces of iron. Balance both tubes to the same shade by adding the ferrous chloride soln dropwise to the appropriate tube and mixing in. *This operation must be carefully done.* When there is no discernible difference in tint, add exactly 1.00 ml of the purpurin indicator to each tube. Mix, then add to each, 2.00 ml of the zirconium soln from a 10 ml buret, and mix in. Do not shake the tubes violently when mixing in reagents; 4 or 5 gentle inversions are sufficient. Observe the color difference, if any, between the two tubes when looking down their length towards a white reflecting surface. If there is no appreciable difference *after 5 min.*, the F content of the sample is negligible. If the color of tube "S" is yellower, the presence of F is indicated. In this case, add additional amounts of the zirconium nitrate to tube "S" until its color matches approximately that of tube "B" (to the nearest 0.5 ml of the zirconium soln). Dilute "S" to mark and mix. Now add to "B" exactly the same total volume of zirconium soln as was added to tube "S," mix, and allow tube to stand for 2 min. for the lake to develop fully. Back-titrate the standard fluoride soln into "B" from a 10 ml buret until the tubes match, frequently mixing, and making nearly to volume as the end point is approached. Add the fluoride in increments of ca 0.1 ml at this stage, and observe the usual precautions of transposing tubes and allowing bubbles to subside when making comparisons. Check the end point by adding 0.1–0.2 ml in excess of the standard fluoride. A distinct overbleach should develop. For a sample weight of 1400 g and the aliquots prescribed above, each ml of standard fluoride soln consumed in the back-titration is equivalent to a F



content on the fruit sample, *removable by the solvent treatment*, of 0.01 grain/lb. Correct the result obtained in the titration by the sample weight ratio. The volume restrictions of a 50 ml Nessler tube will allow the estimation of a spray residue content up to ca 0.05 grain/lb. F. If the calibration mark is exceeded to any great extent in the back-titration, use a 10 ml aliquot of the acid filtrate in tube "S," and make to 25 ml with "blank" soln, correcting the titer of the standard fluoride soln by the appropriate factor.

## 14

## NOTES ON RAPID METHOD

Glass-stoppered Nessler tubes are almost essential with the strong acid prescribed in this determination and are likewise much to be desired in the general method for F with Th and alizarin (9). Analysts familiar with the Th-alizarin back-titration procedure should have no difficulty with the Zr-purpurin titration. With the latter, however, color changes are not so apparent and the titration is less sensitive. However, with careful work, results accurate to .002 grain/lb. may be expected. The indicator color at the prescribed acidity is yellow and the fully laked indicator is orange red. This contrasts with the Th titration where the corresponding range is from a yellowish green to reddish purple. Hence in the present method choice of an end point involves discrimination between varying shades of orange. The addition of the 2.00 ml of Zr soln to tube "B" at the start is merely to provide an intermediate shade of orange to guide the analyst in the amount of Zr to be added to tube "S." Analysts may prefer to work with a redder or yellower end-point shade. In any event, it is urged that a number of titrations be made by adding various quantities of the standard fluoride soln as unknowns to Nessler tubes and carrying through a back-titration as directed above, for the purpose of learning the color changes involved. Pure aqueous solns instead of "blank" may be used with acidities of 20 ml of HCl per 50 ml.

Accuracy of results with the rapid method presupposes complete removal of spray residue F by the solvent process and good accuracy (not necessarily precision) in the titration. These conditions may not always obtain; unless carefully done, the solvent method may not be entirely effective,<sup>7</sup> and results on strip solns containing known quantities of F have tended slightly low. Hence accuracy above 95% is not to be expected with this procedure.

(2) The tentative method for the determination of selenium (*This Journal*, 26, 348) was adopted as official (first action).

## XXX. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

## XXXI. OILS, FATS, AND WAXES

No additions, deletions, or other changes.

## XXXII. PRESERVATIVES AND ARTIFICIAL SWEETENERS

No additions, deletions, or other changes.

## XXXIII. SPICES AND OTHER CONDIMENTS

(1) The method for the determination of total phosphoric acid in vinegar published in *This Journal*, 24, 83, was adopted as official (final action).

(2) The modified Lichthardt method for the detection of caramel in vinegar (*This Journal*, 26, 234) was adopted as tentative.

(3) The tentative method for the determination of color in vinegar removed by fullers' earth (76, p. 481) was deleted.

(4) The following method for the differentiation between vinegar and commercial acetic acid was adopted as tentative:

#### DIFFERENTIATION BETWEEN VINEGAR AND COMMERCIAL ACETIC ACID

##### REAGENTS

(a) *Acetic acid soln.*—To 4 g of acetic acid add 100 ml of  $H_2O$ . (This soln should have a negligible permanganate oxidation number.)

(b) *Sulfuric acid soln.*—Mix equal volumes of  $H_2SO_4$  and  $H_2O$ .

(c) *Potassium iodide soln.*—Dissolve 30 g of KI in 100 ml of  $H_2O$  and filter. Do not use after discoloration with iodine.

##### APPARATUS

For the steam distillation all-glass apparatus is preferable; if this is not available, cover all cork or rubber stoppers with tin or aluminum foil.

##### DETERMINATION

Adjust the vinegar to 4 g/100 ml acidity as acetic with  $H_2O$ . Steam distil 50 ml of adjusted vinegar, maintaining the volume so as to have a residue of 45 ml for 50 ml of distillate. Keep distillate and reagents at  $25^\circ$ . Transfer the 50 ml distillate to a 250 glass-stoppered Erlenmeyer flask. Add 10 ml of the  $H_2SO_4$  and 25 ml of normal permanganate soln. Hold at  $25^\circ$ , preferably in  $H_2O$  bath, for exactly 1 hour. Then immediately add 20 ml of the KI soln and mix well. Titrate the freed iodine with 5 N  $Na_2S_2O_3$  soln. Run blank of the acetic acid soln with the sample and subtract the normal  $KMnO_4$  used from the 25 ml before subtraction of the ml of the 5 N  $Na_2S_2O_3$ .

To obtain the permanganate oxidation number divide by 2 the  $Na_2S_2O_3$  soln used and subtract this from 25. If the permanganate oxidation number is more than 15, repeat, taking half the original quantity of vinegar. Repeat this reduction by half until the ml of normal  $KMnO_4$  soln used is less than 15. To obtain the permanganate oxidation number on the basis of 50 ml, multiply the ml of normal  $KMnO_4$  soln used by the appropriate factor.

If desirable run the permanganate oxidation number on 50 ml of the adjusted vinegar without distillation and also on the undistilled residue made up to 50 ml with  $H_2O$ .

(5) The official method for the determination of salt in prepared mustard (36, p. 474) was deleted (final action).

(6) The method for the determination of salt in prepared mustard, as published in *This Journal*, 24, 703, except that 3–4 grams of sample are specified instead of 5 grams, was adopted as official (final action).

#### XXXIV. SUGARS AND SUGAR PRODUCTS

(1) The official methods for the determination of ash (9, 10, 487) were changed to provide for the use of a sample of appropriate weight for the product being examined.

(2) Hammond's copper equivalents of dextrose, levulose, invert sugar, and mixtures of invert sugar and sucrose containing, respectively, 0.3, 0.4, and 2.0 grams of total sugar (NBS Research Paper RPI 301, p. 589; *J. Research Nat. Bur Standards*, 24, May, 1940; *This Journal*, 25, 674)

were adopted as official (first action). Publication of this table will be postponed until the next revision of *Methods of Analysis, A.O.A.C.*

(3) The method for the determination of moisture in honey by means of a refractometer and the table of refractive index and moisture equivalents of H. D. Chataway (*This Journal*, 25, 99) were adopted as official (final action).

#### XXXV. VEGETABLES AND VEGETABLE PRODUCTS

No additions, deletions, or other changes.

#### XXXVI. VITAMINS

(1) The following assays for the determination of vitamins were adopted as tentative:

(The term "H<sub>2</sub>O" means distilled water in every case.)

##### ASCORBIC ACID (VITAMIN C)

(Applicable to orange, grapefruit, lemon, lime, and tomato juice.)

#### 1

##### REAGENTS

(a) *Metaphosphoric acid—acetic acid stabilizing extracting soln.*—Dissolve with shaking 15 g of stick glacial HPO<sub>3</sub> in 40 ml of glacial acetic acid and 200 ml of H<sub>2</sub>O, dilute to ca 500 ml, and filter rapidly thru a fluted filter into a glass-stoppered bottle. (The HPO<sub>3</sub> slowly changes to H<sub>3</sub>PO<sub>4</sub>, but if stored in refrigerator it remains satisfactory for 7–10 days.)

(b) *Sodium bicarbonate.*—0.05 M NaHCO<sub>3</sub>. Dilute 10 ml of NaHCO<sub>3</sub> with 40 ml of H<sub>2</sub>O.

(c) *Sodium 2,6-dichlorobenzeneindophenol (2,6 dichlorophenolindophenol, sodium salt) soln.*—Dissolve 0.05 g of reagent-grade indophenol dye, which has been stored in a desiccator over soda-lime, in 50 ml of H<sub>2</sub>O to which has been added 42 mg of NaHCO<sub>3</sub>; shake vigorously, and when the dye has dissolved dilute to 200 ml with H<sub>2</sub>O. Filter thru a fluted filter into an amber glass-stoppered bottle. Keep stoppered, out of direct sunlight, and store in refrigerator. (Decomposition products that make the end point indistinct occur in some batches of dry indophenol and also develop with time in the stock soln. Add 5.0 ml of extracting agent containing excess ascorbic acid to 15 ml of the dye reagent. If the reduced soln is not practically colorless, discard and prepare a new stock soln. If the dry dye is at fault, obtain a new specimen.)

(d) *Standard ascorbic acid.*—Use U.S.P. Reference l-ascorbic acid (obtainable from Dr. E. Fullerton Cook, 43rd St. and Woodland Ave., Philadelphia, Pa.). Keep cool, dry, and out of light.

(e) *Indophenol.*—Weigh accurately ( $\pm 0.1$  mg) ca 0.1 g of the U.S.P. Reference l-ascorbic acid, transfer to 100 ml glass-stoppered volumetric flask, and bring to mark (room temp.) with the HPO<sub>3</sub>-acetic acid reagent. Standardize the indophenol reagent at once as follows: Transfer three 2.0 ml aliquots of the standard to each of three 50 ml Erlenmeyer flasks containing 5.0 ml of the HPO<sub>3</sub>-acetic acid reagent. Titrate rapidly with the indophenol reagent from a 50 ml buret until there persists a light but distinct rose-pink color for at least 5 seconds. Each of the titrations should require ca 15 ml of the indophenol reagent, and they should check well within 0.1 ml. In a like manner titrate three blanks composed of 7.0 ml of the HPO<sub>3</sub>-acetic acid reagent plus a volume of H<sub>2</sub>O ca equivalent to volume of indophenol reagent used in direct titrations. After subtracting the average blanks (usually ca 0.1 ml) from the standardization titrations, calculate and express the strength of the indophenol reagent as mg of ascorbic acid equivalent per 1.0 ml of reagent. Standardize the indophenol reagent each day of use with a freshly prepared standard ascorbic acid soln.

## 2

## PREPARATION OF SAMPLE AND DETERMINATION

Prepare juices as directed under 2, p. 335. Add aliquots of at least 100 ml of the prepared juice to equal volumes of the  $\text{HPO}_3$ -acetic acid reagent. Mix, and filter. Titrate 10 ml aliquots as directed for standardization of the indophenol reagent, and make blank determinations for corrections of the titrations as described under 1(e), using the proper volumes of acid reagent and  $\text{H}_2\text{O}$ . Express ascorbic acid as mg/100 ml of original juices.

NOTE: Products containing ferrous iron, originating from defective cans or otherwise, give values in excess of their actual ascorbic acid content by this method. The following is a simple test to ascertain whether ferrous iron is present to an extent that invalidates the test.—Add 2 drops of 0.05%  $\text{H}_2\text{O}$  soln of methylene blue to 10 ml of the freshly prepared sample of juice and the  $\text{HPO}_3$ -acetic acid reagent. A disappearance of the methylene blue color after 5–10 seconds of mixing indicates the presence of interfering substances.

THIOCHROME ASSAY FOR THIAMINE HYDROCHLORIDE (VITAMIN B<sub>1</sub>)

## 1

## REAGENTS AND APPARATUS

(a) *Double-normal sodium acetate*.—Dissolve 275 g of reagent Na acetate in sufficient  $\text{H}_2\text{O}$  to make 1000 ml.

(b) *Bromocresol green pH indicator*.—Dissolve 0.1 g of the indicator by triturating in an agate mortar with 2.8 ml of 0.05 N NaOH, then dilute to a volume of 200 ml with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ .

(c) *Thymol blue pH indicator*.—Dissolve 0.1 g of the indicator by triturating in an agate mortar with 4.3 ml of 0.05 N NaOH, then dilute to a volume of 200 ml with  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ .

(d) *Enzyme soln*.—Prepare on day on which it is to be used a 10% soln ( $\text{H}_2\text{O}$ ) of an enzyme preparation potent in diastatic and phosphorolytic activity.

(e) *Base-exchange silicate*.—Purify an artificially prepared silicate of the base-exchange type, in the form of a granular powder of 50 to 80-mesh size, as follows: Place a convenient quantity (100–500 g) of the base-exchange silicate in a suitable beaker and add sufficient hot 3% acetic acid ( $\text{HC}_2\text{H}_3\text{O}_2$ ) to cover material, and boil 10–15 min., stirring frequently. Allow mixture to settle, and decant the supernatant liquid. Repeat this washing 3 times, then wash in a similar manner 3 times with a hot 25% aqueous soln of KCl (1 part by weight of KCl in 4 volumes of soln), and finally wash with boiling  $\text{H}_2\text{O}$  until the last washing gives no reaction for chloride. Dry the material at ca 100° and store in a well-closed container.

(f) *Base-exchange tube*.—Use a base-exchange glass tube having an over-all length of 200 mm. A reservoir at the upper end, 50 mm in length and 25 mm in diameter, converges into the adsorption tube, which is 5–6 mm internal diameter and ca 140 mm long. At the lower end the tube is drawn into a capillary ca 10 mm long and of such diameter that when the tube is charged the rate of flow will be not more than 1 ml per min. Prepare the tube for use as follows: Place over the upper end of the capillary, with the aid of a glass rod, a pledget of fine glass wool. Add to the adsorption tube an  $\text{H}_2\text{O}$  suspension of 1.0–2.0 g of the purified base-exchange silicate (e), taking care to wash down all the silicate from walls of reservoir. To keep air out of adsorption column, keep a layer of liquid above the surface of the silicate during the adsorption process. The tube may be prevented from draining by placing a rubber cap (filled with  $\text{H}_2\text{O}$  to avoid inclusion of air) over the lower end of the capillary.

(g) *Neutral potassium chloride soln*.—Dissolve 250 g of reagent KCl in sufficient  $\text{H}_2\text{O}$  to make 1000 ml.

(h) *Acid potassium chloride soln*.—Add 8.5 ml of reagent HCl to 1000 ml of the neutral KCl soln (g).

(i) *Sodium hydroxide soln*.—15%. Dissolve 15 g of NaOH in sufficient  $\text{H}_2\text{O}$  to make 100 ml.

(j) *Potassium ferricyanide soln.*—1%. Dissolve 1 g of reagent  $K_3Fe(CN)_6$  in sufficient  $H_2O$  to make 100 ml. Prepare this soln on the day it is used.

(k) *Oxidizing reagent.*—Mix 4.0 ml of the 1%  $K_3Fe(CN)_6$  (j) soln with sufficient 15% NaOH soln (i) to make 100 ml. Use this soln within 4 hours.

(l) *Isobutyl alcohol.*—Use isobutyl alcohol, reagent grade, and redistill in all glass equipment.

(m) *Quinine sulfate stock soln.*—Use quinine sulfate soln to govern the reproducibility of the fluorophotometer. Prepare a stock soln of this reagent by dissolving 10 mg of quinine sulfate in sufficient 0.1 N  $H_2SO_4$  to make 1000 ml. Preserve this soln in light-resistant containers.

(n) *Quinine sulfate standard soln.*—Dilute 1 volume of the quinine sulfate stock soln (m) with 39 volumes of 0.1 N  $H_2SO_4$ . (This soln fluoresces to ca the same degree as does the thiochrome obtained from 1 microgram of thiamine hydrochloride. Preserve this soln in light-resistant containers.

(o) *Thiamine hydrochloride stock soln.*—Weigh accurately 20–25 mg of U.S.P. Thiamine Hydrochloride Reference Standard that has been kept in a desiccator over  $P_2O_5$  for at least 16 hours. Since the reference standard is hygroscopic, take precautions to avoid adsorption of moisture. Dissolve in 20% alcohol adjusted to a pH of 3.5–4.3 with HCl and make up to volume of 1000 ml. Store in cool place in well-closed, light-resistant container.

(p) *Thiamine hydrochloride standard soln.*—From a portion of the stock soln (o) that has been warmed to room temp., transfer to a 100 ml volumetric flask an aliquot containing exactly 100 micrograms of thiamine hydrochloride, and dilute to 100 ml with  $H_2O$  adjusted to a pH of 3.5–4.3 with HCl. (Each ml of this soln contains 1 microgram of thiamine hydrochloride.) Treat dilutions of this soln as directed in 2 with respect to acid digestion, enzyme treatment, adsorption, and elution from the base exchange silicate.

## 2

## PREPARATION OF ASSAY SOLUTION

Take such a quantity of material for the assay that the ratio of the volume of 0.1 N  $H_2SO_4$  used for the extraction of the quantity of sample is at least 15 to 1, and the content of thiamine is equivalent to 30–100 micrograms of thiamine hydrochloride. Place the accurately weighed material in 65 ml of 0.1 N  $H_2SO_4$  contained in a 100 ml centrifuge tube and digest on a steam bath, with frequent mixing, for 30 min. If the liquid is not distinctly acid to the thymol blue pH indicator, add sufficient 10%  $H_2SO_4$  to make it acid. Cool, and adjust the pH to 4–4.5 by the addition of the double-normal Na acetate soln, using bromocresol green pH indicator as an external indicator in conjunction with a spot plate. Add 5 ml of the enzyme soln, mix, and incubate at 45–50° for 3 hours. Cool, centrifuge mixture until supernatant liquid is clear or practically so, and quantitatively transfer supernatant liquid to a 100 ml volumetric flask. Wash residue by centrifuging with 10 ml, then with 5 ml of 0.1 N  $H_2SO_4$ . Add washings to supernatant liquid and dilute to 100 ml with  $H_2O$ .

Pass thru the prepared base-exchange tube an aliquot of the soln estimated to contain 5–10 micrograms of thiamine, and wash tube with three 5 ml portions of boiling  $H_2O$ , taking care to prevent the surface of the liquid from falling below the surface of the silicate.

Elute the thiamine from the base-exchange silicate by passing successively thru the tube small portions of the hot acid KCl soln. Collect the first 15 ml of the liquid (eluate) in a glass-stoppered, 25 ml volumetric flask, cool, and dilute to a volume of 25 ml with acid KCl soln. This constitutes the assay soln.

## 3

OXIDATION OF THIAMINE TO THIOCHROME AND  
MEASUREMENT OF FLUORESCENCE

Determine the thiamine content of the oxidized assay soln by comparing the intensity of fluorescence of an extract of this soln exposed to ultraviolet rays ranging

from 350  $m\mu$  to 400  $m\mu$  with that from the oxidized thiamine hydrochloride standard soln. The intensity of the fluorescence is proportional to the quantity of thiamine present and may be measured with the aid of various instruments.

To oxidize thiamine to thiochrome add to quantities of the assay soln and of the similarly treated thiamine hydrochloride standard soln containing 0.10–2.0 micrograms of thiamine, sufficient acid KCl to produce a volume of 5 ml. Then add with mixing 3 ml of oxidizing soln. Immediately thereafter add 13 ml of isobutyl alcohol and shake vigorously for at least 1½ min. Centrifuge the mixture at a low speed until a clear supernatant soln is obtained. Measure in a fluorometer the intensity of fluorescence of the isobutyl alcohol soln directly if clear, or if cloudy, after shaking with 2 g of anhydrous  $\text{Na}_2\text{SO}_4$ . Compare with this the intensity of fluorescence produced after oxidation of the properly prepared thiamine hydrochloride standard soln. Use quinine sulfate standard soln to govern the reproducibility of the instrument, and make correction for fluorescence produced by substances other than thiamine by determining the intensity of fluorescence of thiamine hydrochloride standard soln, and assay solns treated as described above, but with 15% NaOH soln replacing the oxidizing reagent.

(The thiochrome assay for thiamine hydrochloride is applicable to a number of materials, but cannot be relied upon when certain interfering substances are present.) In the latter case, use the tentative A.O.A.C. biological assay for thiamine hydrochloride (*This Journal*, 24, 150).

#### MICROBIOLOGICAL ASSAY FOR NICOTINIC ACID (NIACIN) OR NICOTINAMIDE (NIACINAMIDE)

##### REAGENTS

(a) *Test soln of material to be assayed.*—Place sufficient material, accurately weighed, to represent 0.02 to 0.1 mg of nicotinic acid, in a 300 ml flask, add 100 ml of normal  $\text{H}_2\text{SO}_4$ , and mix thoroly. Heat mixture in autoclave at 15 lbs. pressure (121.5°) for 30 min., cool, add NaOH T.S. to produce a pH of 6.8, and add  $\text{H}_2\text{O}$  to make a volume such that 1 ml contains ca 0.1 microgram nicotinic acid.

(b) *Standard nicotinic acid soln.*—Accurately weigh 50 mg of U.S.P. Nicotinic Acid Reference Standard and add sufficient alcohol to make 500 ml. Store this stock soln in refrigerator. Prepare the standard soln by diluting 1 ml of the stock soln, which has been warmed to room temp., with sufficient  $\text{H}_2\text{O}$  to make 1000 ml, representing 0.1 microgram of the reference standard in each ml of soln. Prepare fresh standard soln for each assay.

(c) *Basal medium stock soln.*—Sufficient for 50 tubes.

	ml
Acid-hydrolyzed casein soln.....	25
Tryptophane soln.....	50
Cystine soln.....	100
Adenine-guanine-uracil soln ..	5
Thiamine hydrochloride soln .....	0.5
Calcium pantothenate soln.....	0.5
Pyridoxine hydrochloride soln.....	1
Riboflavin soln.....	1
p-Aminobenzoic acid soln.....	0.5
Biotin soln.....	2
Salt soln A*.....	2.5
Salt soln B†.....	2.5
	grams
Anhydrous dextrose.....	5
Sodium acetate.....	3

\* Dissolve 25 g of  $\text{KH}_2\text{PO}_4$  and 25 g of  $\text{K}_2\text{HPO}_4$  in sufficient  $\text{H}_2\text{O}$  to make 250 ml of soln.

† Dissolve 10 g of  $\text{MgSO}_4$ , 0.5 g of reagent NaCl, 0.5 g of  $\text{FeSO}_4$ , and 0.5 g of  $\text{MnSO}_4$  in sufficient  $\text{H}_2\text{O}$  to make 250 ml.

Mix the ingredients, adjust soln to pH of 6.8, and add sufficient H<sub>2</sub>O to make 250 ml.

(d) *Acid-hydrolyzed casein soln.*—Mix 100 g of vitamin-free casein with 500 ml of constant-boiling HCl (ca 20% HCl) and reflux mixture for 8 hours. Remove the HCl from mixture by distillation under reduced pressure until a thick sirup remains. Dissolve sirup in H<sub>2</sub>O and again concentrate mixture in same manner. Redissolve the resulting sirup in H<sub>2</sub>O, adjust the soln to pH of 3.0 with NaOH T.S., and add sufficient H<sub>2</sub>O to make 950 ml. Add to soln 20 g of activated charcoal, and stir for an hour, then filter. Repeat the treatment with activated charcoal if the filtrate does not appear straw colored to colorless. Adjust the pH of the filtrate to 6.8 and add sufficient H<sub>2</sub>O to bring the volume to 1000 ml. Store this soln under toluene in refrigerator.

(e) *Cystine soln.*—Dissolve 1 g of l-cystine in 20 ml of 10% HCl and add sufficient H<sub>2</sub>O to make 1000 ml. Store soln under toluene in refrigerator not below 10°.

(f) *Tryptophane soln.*—Dissolve 1 g of l-tryptophane in 5–6 ml of 20% HCl and add sufficient H<sub>2</sub>O to make 1000 ml. Store soln under toluene in refrigerator.

(g) *Adenine-guanine-uracil soln.*—Dissolve 0.1 g of each adenine sulfate, guanine hydrochloride, and uracil with the aid of heat in 5 ml of 20% HCl, and add sufficient H<sub>2</sub>O to make 100 ml. Store soln in refrigerator.

(h) *Thiamine hydrochloride soln.*—Prepare a soln containing 0.1 mg/ml by dissolving crystalline thiamine hydrochloride in 25% alcohol adjusted to a pH of 3 with HCl. Store soln in refrigerator.

(i) *Calcium pantothenate soln.*—Prepare a soln containing 0.1 mg/ml by dissolving crystalline calcium pantothenate in neutral 25% alcohol. Store soln in refrigerator.

(j) *Pyridoxine hydrochloride soln.*—Prepare a soln containing 0.1 mg/ml by dissolving crystalline pyridoxine hydrochloride in 25% alcohol. Store soln in refrigerator.

(k) *p-Aminobenzoic acid soln.*—Prepare a soln containing 0.1 mg/ml by dissolving crystalline p-aminobenzoic acid in 25% alcohol. Store soln in refrigerator.

(l) *Riboflavin soln.*—Prepare a soln containing 0.1 mg/ml by dissolving crystalline riboflavin in 0.02 N acetic acid. Store the soln, protected from light, in refrigerator.

(m) *Biotin soln.*—Prepare a soln containing 0.1 microgram/ml by dissolving crystalline biotin (free acid) in 50% alcohol. Store soln in refrigerator.

(n) *Stock culture of Lactobacillus arabinosus 17-5.\**—To 5 ml of yeast extract soln in 95 ml of H<sub>2</sub>O, add 1 g of anhydrous dextrose and 1.5 g of agar, and heat mixture on steam bath until agar has dissolved. Add ca 10 ml portions of the hot soln to test tubes, plug tubes with non-absorbent cotton, sterilize in autoclave at 15 lbs. pressure (121.5°) for 20 min., and allow to cool in an upright position. Prepare stab cultures, using a pure culture of *Lactobacillus arabinosus 17-5,\** incubate for 16–24 hours at any selected temp. between 30° and 37° held constant to within  $\pm 0.5^\circ$ , and finally store in refrigerator. Prepare a fresh stab of stock culture every week and do not use for inoculum if the culture is more than 2 weeks old.

(o) *Culture medium.*—To each of a series of tubes containing 5 ml of the basal medium stock soln add 5 ml of H<sub>2</sub>O containing 2 micrograms of nicotinic acid. Sterilize in autoclave at 15 lbs. pressure (121.5°) for 20 min.

(p) *Inoculum.*—Make a transfer of cells from the stock culture of *Lactobacillus arabinosus 17-5* to a sterile tube containing 10 ml of culture medium. Incubate this culture for 16–24 hours at any selected temp. between 30° and 37° held constant to within  $\pm 0.5^\circ$ . Make a transfer of one drop from this tube to another sterile tube of

\* Pure cultures of *Lactobacillus arabinosus 17-5* may be obtained as culture No. 8014 from the American Type Culture collection, Georgetown University Medical School, Washington, D. C.

culture medium and incubate for 16–24 hours at any selected temp. between 30° and 37°. Under aseptic conditions centrifuge the culture and decant the supernatant liquid. Prepare the inoculum by suspending the cells from the culture in 10 ml of sterile isotonic soln of NaCl. If assays are to be made on each of several successive days, the inoculum may be prepared by successive daily transfers in the culture medium for a period not exceeding 1 week.

#### ASSAY PROCEDURE

Prepare standard nicotinic acid tube as follows: To duplicate tubes, 16×150 mm in size, add 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ml, respectively, of the standard nicotinic acid soln. To each of these tubes add 5 ml of basal medium stock soln (c) and sufficient H<sub>2</sub>O to bring the volume in each tube to 10 ml.

Prepare tubes containing the material to be assayed as follows: To duplicate tubes add, respectively, 1.0, 2.0, 3.0, and 4.0 ml of the test soln of the material to be assayed. To each of these tubes add 5 ml of basal medium stock soln and sufficient H<sub>2</sub>O to bring volume in each tube to 10 ml.

After thoro mixing, plug tubes of two series mentioned above with non-absorbent cotton, and autoclave at 15 lbs. pressure (121.5°) for 20 min. Cool, aseptically inoculate each tube with 1 drop of inoculum, and incubate for 72 hours at any selected temp. between 30° and 37°, held constant to within ±0.5°. Contamination of the assay tubes with any organisms other than *Lactobacillus arabinosus* invalidates the assay.

Transfer contents of each tube to suitable container, using ca same quantity of H<sub>2</sub>O in each instance for rinsing. Titrate contents of each flask with 0.1 N NaOH, using bromothymol blue as indicator, or to pH of 6.8 measured electrometrically.

#### CALCULATION

Prepare a standard curve of the nicotinic acid standard titrations by plotting the average of the titration values expressed in 1 ml of 0.1 N NaOH for each level of nicotinic acid standard soln used, against micrograms of nicotinic acid contained in the respective tubes. From this standard curve determine by interpolation the nicotinic acid content of the test soln in each duplicate set of tubes. Discard any values that show more than 0.4 or less than 0.05 microgram of nicotinic acid in each tube. Calculate nicotinic acid content in each ml of test soln for each of the duplicate sets of tubes, and that of the test material from the average of the values obtained from not less than 3 sets of these tubes that do not vary by more than ±10% from the average. If the titration values of two or more of the duplicate sets of tubes containing the test soln fall below the titration values of the Nicotinic Acid Standard tubes containing 0.05–0.4 mg of nicotinic acid, the nicotinic acid content of the test soln is too low to permit calculation of nicotinic acid content of the test material. Titration values exceeding 2 ml for the tubes of the standard nicotinic acid soln series containing 0.0 ml of the soln indicate the presence of an excessive amount of nicotinic acid in the basal medium stock soln and invalidate the assay.

#### CAROTENE

##### *Chromatographic Method*

#### 1

##### REAGENTS

(a) *Alcoholic potash*.—Dissolve 12 g of KOH in 100 ml of 95% ethyl alcohol.

(b) *Petroleum benzin*.—Use petroleum benzin, boiling point 30–70°, or Skellysolve F, boiling point 86–170°F (30–70°C.).

(c) *Methanol*.—90%. Dilute 100 ml of water in a 1 liter flask to volume with absolute methanol.



(d) *Calcium hydroxide*.—Use ordinary commercial hydrated lime that has been sifted thru a 48–65-mesh sieve.

## 2

## PREPARATION OF ADSORPTION COLUMN

Prepare the adsorption column as directed in *This Journal*, 25, 886, except to use the  $\text{Ca}(\text{OH})_2$  instead of  $\text{MgO}$  and omit cooling the column with ice water.

## 3

## EXTRACTION OF PIGMENTS

In the extraction of pigments, avoid the use of methods in which heating is used because heating produces neo-beta carotene.<sup>1</sup>

(a) *Fresh materials, butter, egg yolk, and fresh or dried fecal material*.—Chop the fresh material into pieces ca  $\frac{1}{4}$ " long and weigh 10 g into the chamber of a Waring blender or similar type of apparatus. Add 150–200 ml of the alcoholic KOH and grind for 5 min. Extract with petroleum benzin and wash with methyl alcohol as directed in 61, p. 369.

(b) *Dried hays, grass, and dehydrated leafy vegetables*.—To 5–25 g of material, depending on carotene content, add 150–200 ml of alcoholic KOH and let stand overnight in refrigerator. Then grind in a Waring blender or similar type apparatus and extract as directed in 3(a).

(c) *Fresh sweet potatoes*.—Proceed as directed for other fresh materials, 3(a), except to use 95% ethyl alcohol in place of the alcoholic KOH.

(d) *Dehydrated fruits or vegetables high in sugar, such as dried apricots, carrots, or sweet potatoes*.—Weigh a 5–10 g sample, add 50–100 ml of water, and allow to stand overnight in refrigerator. Then proceed as directed in 3(a).

## 4

## SEPARATION OF PIGMENTS

Separate the chromatographic pigments as directed in the method for yellow corn published in *This Journal*, 25, 887. With most materials, the bands of pigments found in the columns starting from the top are as follows:

(a) *Impurity A*.—May consist of several bands of yellow, red, or brownish yellow pigments.

(b) *Carotenoid X*.—A light orange pigment just above the beta carotene band. This pigment separates late in the analysis.

(c) *Beta carotene*.—A wide reddish orange band.

(d) *Neo-beta carotene*.—A yellow-orange band immediately below the beta carotene band.

(e) *Alpha carotene*.—An orange band that is not present in most materials in appreciable amounts.

Another small band is sometimes present below the alpha carotene band. For all practical purposes it may be removed with the alpha carotene. Sometimes carotenoid X separates from the other pigments very slowly. Washing the column with petroleum benzin containing 1–5% acetone is often helpful.

(2) The following modifications of the tentative method for the determination of crude carotene in hay and dried plants (61, p. 369) were adopted to extend the utilization of the method to other materials:

(a) *Fresh green materials*.—For samples to be analyzed soon after gathering (1–2 hours), place 100 g of material in large evaporating dish, soak in 100 ml of 95% ethyl alcohol for 5 min., and cut up with scissors. Add 100 g of clean white sand, free of organic matter, and grind until a uniform mixture is obtained. For samples necessi-

<sup>1</sup> *Ind. Eng. Chem.*, In press (1943).

tating shipment or delay in analysis, place ca 100 g of material in tared fruit jar with weighed quantity of alcohol or methanol. Seal jar by using jar rubber under lid. Upon arrival at laboratory, weigh jar and contents, and subtract weight of jar and alcohol from total weight to obtain weight of sample.

Pour contents of jar into an evaporating dish, cut up with scissors, and grind with sand as directed above. Decant liquid from solid part thru cheese cloth and make up to volume. Weigh solids. Take aliquots of both solid and liquid, equivalent to 5 g of the fresh untreated material and mix together for carotene analysis. Saponify sample by boiling for 30 min. in 50 ml of 12% alcoholic KOH. Cool, add 50 ml of petroleum benzin, and decant liquid into separatory funnel. Transfer residue to mortar and grind with pestle, first with 15 ml portion of petroleum benzin and then with mixture of 5 ml of 95% ethyl alcohol and 15 ml of petroleum benzin until no further color is extracted. Then proceed as directed in 61, p. 369.

(b) *Fresh green materials that have been preserved in methanol or ethyl alcohol and only crude carotene on dry basis is desired.*—Pour off as much of the alcohol as possible. Grind solid portion in food chopper and place in tightly sealed jar to prevent evaporation. Weigh out 5 g of the solid material and proceed as directed in (a). Determine dry matter in portion of sample.

(c) *Fresh carrots and apricots.*—Grind material in food chopper. Weigh 5 g of sample and reflux with alcoholic KOH as directed in 61, p. 369, except to triturate the residue with petroleum benzin and alcohol until no more color is removed.

(d) *Fresh sweet potatoes.*—Proceed as directed in 61, p. 369, except to reflux with 95% ethyl alcohol instead of alcoholic KOH.

(e) *Dehydrated fruits and vegetables containing high amounts of sugar* (carrots, sweet potatoes, apricots, etc.).—To 2–5 g sample, add 20–50 ml of  $H_2O$  and allow to stand overnight in refrigerator. Then proceed as directed in 61, p. 369. (Use of a Waring blender or similar type apparatus is helpful in the extraction of these samples.)

(f) *High lycopene-containing materials* (tomatoes and watermelons).—

#### REAGENTS

*Magnesium carbonate.*—Use U.S.P. light  $MgCO_3$  that will adsorb less than 5% of carotene when tested by the following method:

Place ca 1 g in a tube as directed below and pass thru it a soln of purified carotene (*This Journal*, 24, 860), containing 1.0–1.5 p.p.m. of carotene. Wash with the petroleum benzin and determine the carotene in the filtrate. If the  $MgCO_3$  is too retentive of carotene, try another lot.

#### PREPARATION OF COLUMN

Place 1 g of the  $MgCO_3$  in a glass tube 5–8 mm wide and ca 15 mm tall, constricted at one end and plugged with a wad of cotton. Apply suction and pack the  $MgCO_3$  firmly but not too tightly with a cork having a smooth surface and attached to a glass rod.

#### DETERMINATION

Weigh 5 g of sample and obtain the crude carotene as directed in 61, p. 369. Dilute to 200 ml. Evaporate 50 ml aliquot in vacuo to ca 10 ml. Then run thru a column of  $MgCO_3$ . Wash column with petroleum benzin until all carotene is washed thru. (A red band of lycopene will pass slowly down the column. Be careful that none of this pigment is washed thru the column.) Dilute soln that passes thru column to appropriate volume and determine the amount of crude carotene as directed in 61, p. 369.

(g) *Canned foods*.—Pour contents of can onto piece of cheese cloth and allow liquid to drain. Grind solids in food chopper and proceed as directed in 61, p. 369.

(h) *Butter and other fats, egg yolks, and liver*.—Weigh 5 g sample and proceed as directed in 61, p. 369.

(i) *Blood plasma*.—Weigh 10–30 g of blood plasma and reflux with 25–50 ml of alcoholic KOH for 15 min. Proceed as directed in 61, p. 369.

### XXXVII. WATERS, BRINE, AND SALT

(1) The tentative method of reporting results on waters and brine (81, p. 543) and the table of combining weights and their reciprocals were made official (first action).

(2) The method for the determination of fluorides in water (22, p. 529, and corrected in *This Journal*, 25, 101) was adopted as official (final action).

(3) Tentative methods I and II for the determination of iodides in salt (100–105, pp. 547–548) were deleted.

(4) As modified by the associate referee, the tentative Elmslie-Caldwell method for the determination of iodides in mineral mixed feeds (58, p. 368) was adopted as official (first action) for the determination of iodine in salt. This method follows:

#### IODINE IN SALT

Take 25 ml aliquots of a 20% soln of iodized salt, add 5 g of  $\text{Na}_2\text{CO}_3$ , cover with watch-glass, and boil gently for 10 min. Transfer contents to 18 cm filter paper and wash with boiling  $\text{H}_2\text{O}$ , catching filtrate and washings in 500 ml Erlenmeyer flask (soln should total ca 300 ml). Neutralize to methyl orange with 85%  $\text{H}_3\text{PO}_4$ , and add 1 ml in excess.

Add excess of Br water and boil soln gently until colorless, and then 5 min. longer. Add a few crystals of salicylic acid and cool to ca  $20^\circ$ . Add 1 ml of 85%  $\text{H}_3\text{PO}_4$  and ca 0.5 g of KI and titrate I with 0.005  $N$   $\text{Na}_2\text{S}_2\text{O}_3$  in the usual way, using starch soln (3(e), 44) as indicator.

Standardize the  $\text{Na}_2\text{S}_2\text{O}_3$  soln by measuring into a 500 ml Erlenmeyer exactly 25 ml of a standard soln containing 0.1308 g of KI per liter, adding 300 ml of  $\text{H}_2\text{O}$  and 5 g of  $\text{Na}_2\text{CO}_3$ , neutralizing, and proceeding as directed above, beginning "Add excess of Br water. . . ."

(It is advisable to standardize the  $\text{Na}_2\text{S}_2\text{O}_3$  soln same day determinations are conducted.)

### XXXVIII. RADIOACTIVITY

No additions, deletions, or other changes.

### XXXIX. DRUGS

(1) The title of the official method for the determination of barbital and phenobarbital (44, 45, p. 574) was changed to read "Barbiturates," and to 44(b) there was added the following statement: "(Chloroform alone may be used except with nostal and pernoston)."

(2) The microchemical tests for the determination of choline published in *This Journal*, 26, 96, were adopted as tentative.

(3) The microchemical test for the determination of sulfadiazine published in *This Journal*, 26, 97, was adopted as tentative.

(4) The iodination method for the determination of phenolphthalein in emulsions published in *This Journal*, 26, 312, was adopted as tentative.

(5) The spectrophotometric method for the determination of quinine published in *This Journal*, 26, 240, was adopted as tentative.

(6) The following spectrophotometric method for the determination of quinacrine was adopted as tentative:

#### QUINACRINE

##### APPARATUS

(a) *Spectrophotometer or photoelectric photometer*.—Having a filter with a peak transmittance at 524 m $\mu$ .

(b) *Matched 1 cm absorption cells*.

##### REAGENTS

(a) *Hydrochloric acid*.—Approximately 0.1 N.

(b) *Quinacrine hydrochloride*.—Determine purity by U.S.P. assay or nitrogen by Kjeldahl method, 21, p. 26.

(c) *Standard soln*.—2.5 mg of quinacrine hydrochloride per 100 ml of 0.1 N HCl.

##### DETERMINATION

Accurately weigh or measure a quantity of sample containing ca 100 mg of quinacrine hydrochloride and transfer to 1000 ml volumetric flask. Add ca 100 ml of the HCl and heat on steam bath until the quinacrine hydrochloride has dissolved. Cool, and dilute to volume with the HCl.

Filter soln if not perfectly clear. Pipet an aliquot containing 2–3 mg of quinacrine hydrochloride into 100 ml volumetric flask and fill to mark with the HCl. Determine the absorption (E) of the standard soln and also relative to a blank of 0.1 N HCl at 425 m $\mu$ .

$$\text{Mg of quinacrine hydrochloride in aliquot} = \frac{E_{\text{sample}}}{E_{\text{standard}}} \times 2.5.$$

(7) The following methods were made official (final action):

Monobromated camphor, Method II (51, p. 576).

Pilocarpine hydrochloride (95, p. 589).

Calomel in calomel ointment (192, p. 620).

Hypophosphites in sirup, Methods I and II (180, 181, p. 617).

Salicylic acid in presence of other phenols (38, p. 572).

Tetrachloroethylene in mixtures (135, p. 604).

(8) The following tentative methods were adopted as official (first action):

Acetophenetidin and caffeine (16, p. 565).

Acetylsalicylic acid, acetophenetidin, and caffeine (32, p. 570).

Bismuth compounds in tablets (178, p. 617).

Calcium gluconate (179, p. 617).

Effervescent potassium bromide with caffeine (202, p. 623).

Iodine (183, p. 618).

Mandelic acid (154, p. 610).

Oil of chenopodium (208, p. 625).

Phenolphthalein in chocolate preparations (162, p. 613).

Sulfanilamide (168, p. 614).

Theophylline (107, p. 593).

## **XL. MICROBIOLOGICAL METHODS**

No additions, deletions, or other changes.

## **XLI. MICROCHEMICAL METHODS**

No additions, deletions, or other changes.

## **XLII. STANDARD SOLUTIONS**

(1) The method for the preparation of standard hydrochloric acid solution from constant boiling acid (*This Journal*, 25, 110) was adopted as official (final action).

(2) The method for standardizing sodium hydroxide solutions by means of constant boiling hydrochloric acid (*This Journal*, 25, 112) was adopted as official (final action).

## **COSMETICS**

(1) The tentative method for the determination of salicylic acid in hair lotions (*This Journal*, 25, 112; and as revised, *Ibid.*, 26, 355) was adopted as official (first action).

(2) The method for the determination of sulfides in depilatories published in *This Journal*, 25, 113, was adopted as official (final action).

(3) The two methods, benzoquinone and acetylation, for the determination of paraphenylenediamine in hair dyes, published in *This Journal*, 25, 113, were adopted as official (final action).

(4) The methods for the determination of D&C Orange No. 3 and D&C Yellow No. 7 published in *This Journal*, 25, 114, were adopted as official (final action).

(5) The following method for the determination of pure dye in D&C Red Nos. 8 and 31 was adopted as tentative:

### **PURE DYE IN D&C RED NO. 8 AND D&C RED NO. 31**

In a wide-mouthed Erlenmeyer flask dissolve 0.2 g of sample in 5 ml of  $H_2SO_4$ . Dilute with 100 ml of  $H_2O$  and add sufficient 30% NaOH to leave soln slightly acid. Add 15 g of Na bitartrate and heat to dissolve the buffer. Add ca 125 ml of 95% alcohol, heat to boiling, and titrate slowly, especially towards end, with  $TiCl_3$  to a yellow end point.

1 ml of 0.1 N  $TiCl_3$  = 0.00997 g of D&C Red No. 8

1 ml of 0.1 N  $TiCl_3$  = 0.007783 g of D&C Red No. 31

(6) The method for the determination of halogens in halogenated fluoresceins published in *This Journal*, 25, 757, was adopted as tentative after the following change had been made in the wording for clarification. Change the last two sentences of paragraph I(1) to read: "Continue to add the  $KNO_2$  until practically all the  $MnO_2$  is reduced. If a few particles of  $MnO_2$  resist the action of the  $KNO_2$ , do not attempt to destroy these,

but immediately add the dilute  $\text{KMnO}_4$  in 1 ml portions until soln becomes pink."

(7) The method for the determination of alizarin in madder lake published in *This Journal*, 25, 956; 26, 242, was adopted as official (first action).

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No report was given by the Committee to Confer with American Public Health Association on Standard Methods of Milk Analysis.

## REPORT OF REPRESENTATIVES ON BOARD OF GOVERNORS OF CROP PROTECTION INSTITUTE

This report covers the years 1942 and 1943.

The Crop Protection Institute has continued to function as in former years, though with fewer projects. Most of the research pursued has been closely related to the war emergency. Many of the projects sought substitutes for insecticides and fungicides that are no longer available and the devising of means for the more efficient use of materials that are still available through the use of new spreaders and adherents.

The demand for food and the interest in its products through many new channels have stimulated and given an impetus to research aimed at crop protection and security. The result of this awakened interest is likely to continue for some years and we hope that it may lead to the discovery of new materials and methods that will prove very worth-while in practice.

The world-wide contacts and interchange of materials which the war has caused are sure to bring the introduction of a variety of new plant and animal diseases and pests, which will flourish in their new environments and present either new or enlarged problems, the solution of which will require the active cooperation of chemists with the entomologists, plant physiologists, and plant pathologists.

The program of the Crop Protection Institute has continued to emphasize the importance of finding insecticides and fungicides that are efficient, cheap, and harmless to man and animals. Some by-products that have resulted from the manufacture of war goods will no doubt have value in crop growth and protection. These complex objectives require a wide range of research activities.

The program of the Institute has been interrupted in some instances and slowed down in others by the shortage of technical men. This condition will continue for the duration of the war. The Institute is conducting the research projects listed below for the following firms:

1. Albi Chemical Company.—Tests as fungicides of copper compounds, and modifications of them, now extensively used for mildew-proofing fabrics.

Armour and Company.—Tests of dried blood albumin as an adjunct for sprays and as an emulsifying agent for oil sprays. It has been found that 1 pound of dried blood to 100 gallons of spray enhances the effectiveness of nicotine spray. Dried blood in combination with fullers' earth increases the adhesiveness of insoluble materials in sprays.

3. Carbide and Carbon Chemical Corporation.—Fungicide investigations, including test of three new compounds for the control of apple scab. Two of these have given very promising results. Also insecticide investigations that include tests of toxicants and repellents for fly sprays. Significant advances and promising data have been secured.

4. Floriden Company.—Testing various combinations and methods for combining fullers' earth with fungicides and insecticides for increasing their effectiveness. Results indicate a new way has been discovered.

5. General Chemical Company.—Tests for some new organic compounds as fungicides. Work was conducted in North Carolina, Colorado, Mississippi, Ohio, and New York.

6. Mathieson Alkali Works, Inc.—Tests of new residues as fungicides and household fly sprays.

7. Röhm and Haas Company.—Studies of new cuprous and organic compounds as fungicides.

8. The Solvay Process Company.—Tests of new products for use both as fungicides and insecticides.

9. U. S. Industrial Chemicals, Inc.—Testing the growth-promoting properties of an organic material derived from molasses.

The Institute also has conducted a considerable amount of preliminary exploratory work that could not be set up as a definite project. Much of this work has been done under the supervision of Dr. O'Kane in his laboratory.

The Board of Governors of the Institute would appreciate suggestions from this Association or its members as to the nature of its activities and fields where it might serve.

H. J. PATTERSON  
W. H. MACINTIRE

Approved.

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## REPORT OF SECRETARY-TREASURER

By W. W. SKINNER

During the two years since our last meeting the following members of this Association have died:

John S. Abbott  
Theodore F. Pappe  
Walter S. Frisbie

G. S. Spencer  
Robert Harcourt  
Richard F. Jackson

Samuel Palkin  
Fletcher P. Veitch  
Robert H. Kerr

Melvin Pingree  
Frank L. Wollard  
George H. Adams

The Committee on Necrology will report more fully on these former members and their obituaries.

At a meeting of the Executive Committee held at the Cosmos Club Tuesday evening, October 26, there were present J. W. Sale, L. B. Broughton, G. G. Frary, J. O. Clarke, W. H. Ross, and W. W. Skinner, members of the Committee; H. A. Lepper, Chairman of the Editorial Committee and Editor of *The Journal*; and Miss Lapp, Assistant Secretary. G. H. Marsh was unable to come to Washington for the meeting.

It was suggested that it would be well for the Auditing Committee to be appointed in advance of the meeting so that the members might have an opportunity to go over the auditor's report carefully and confer with him if necessary in regard to any items.

After a discussion as to whether the stock of Journals in the store room should be counted it was moved, seconded, and carried that this be done in view of the fact that Dr. Skinner is retiring and turning the property of the Association over to the new Secretary-Treasurer.

It was moved, seconded, and carried that every three years the accounts receivable be broken down and reported in detail and that this be done this year for the accounts of 1941-1943.

The 1945 revision of *Methods of Analysis*, A.O.A.C., was considered and Mr. Lepper also discussed the affairs of *The Journal* and matter of reprints. No definite changes were made in regard to policy in connection with either publication.

The financial accounts for the year ending September 30 were audited by Lionel Farr of the firm of Snyder, Farr & Company. The following financial statement was read at the meeting of the Executive Committee and approved.

#### STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR FISCAL YEAR ENDED SEPTEMBER 30, 1943

##### CASH BALANCE, OCTOBER 1, 1942

Cash, Lincoln National Bank.....	\$4,063.21	
Cash, Montgomery Mutual Building & Loan Association	1,203.78	
Cash on hand.....	12.60	\$ 5,279.59

##### CASH RECEIPTS

Sale of Methods.....	\$5,760.88	
Sale of Journals.....	5,663.62	
Sale of Wiley's Principles.....	6.60	
Sale of Reprints.....	178.80	
Sale of advertising.....	449.70	12,059.60



For books ordered (for others).....	755.73
Treasury bond redeemed.....	1,000.00
Interest on investments (bonds).....	392.50
Interest on Building & Loan Association account.....	48.15
Miscellaneous receipts.....	3.65
<i>Total cash for which to account.....</i>	<i>\$19,539.22</i>

## CASH DISBURSEMENTS

Salaries.....	\$1,807.50	
Social security taxes.....	12.38	
Postage.....	330.17	
Association and meeting expense.....	123.52	
Auditing.....	150.00	
Refunds.....	13.00	
Traveling expense.....	150.00	
Printing and binding:		
Reprints.....	\$ 694.61	
Journals.....	4,490.68	5,185.29
War bonds.....	5,920.00	
Books ordered (purchased for others).....	781.17	
<i>Total disbursements.....</i>		<i>14,473.03</i>
		<u><u>\$ 5,066.19</u></u>

## CASH BALANCE, SEPTEMBER 30, 1943

*Represented by:*

Cash on hand (petty cash).....	\$ 57.43	
Cash on deposit, Lincoln National Bank.....	3,756.83	
Cash on deposit, Montgomery Mutual Building & Loan Association.....	1,251.93	\$ 5,066.19

STATEMENT OF INCOME AND EXPENSE  
FOR FISCAL YEAR ENDED SEPTEMBER 30, 1943

## INCOME

Advertisements.....	\$ 449.70	
Journals.....	5,953.01	
Methods.....	6,636.41	
Wiley's Principles.....	12.75	
Reprints.....	178.80	\$13,230.67
Interest on investments (bonds).....	552.50	
Interest on Building & Loan Association account.....	48.15	
<i>Total income.....</i>		<i>\$13,831.32</i>

## EXPENSES

Discounts and allowances and refunds.....	\$1,540.06
Printing and binding.....	7,054.88
Salaries.....	1,807.50
Postage.....	328.42
Association and meeting expense.....	229.51
Auditing.....	150.00
Exchange.....	20.09
Social security taxes.....	12.38
Miscellaneous expense.....	24.94
<i>Total expenses.....</i>	<u>11,167.78</u>
<i>Excess of income over expenses, to surplus.....</i>	<u>\$ 2,663.54</u>

The A.O.A.C. is not a money making organization, and it is not in my opinion necessary or desirable to accumulate a large surplus fund. Therefore, one matter for the new board to consider seriously relates to its finances. When surplus funds were accumulating faster than seemed desirable a few years ago we reduced the cost of *Methods of Analysis* to members from \$5.00 to \$4.00. I do not advocate that the price of the book be further reduced; I think it would tend to cheapen the book to do so, but presumably, unless conditions at the time of the next issue are very different from what they were before, this publication will continue to be a definite source of surplus income for the Association.

The Association is a public service corporation, and is incorporated under the Laws of the District of Columbia, but we are not taxed because we are a non-profit public service institution. However, apparently the auditor thinks we are not relieved from the fairly recent requirement of a liability to the Unemployment Compensation Board for the District of Columbia. He has recommended that we learn our status in relation to this law.

I shall make no attempt to tell you in detail of the pleasures or the woes that have been a part of the office of Secretary-Treasurer for a period of twenty odd years. There have been some difficulties, which you can find if you look through the reports in *The Journal*. There have also been some very distinct pleasures, and therefore I lay down this office with some regret, but also with a very distinct feeling of having performed a service in which I feel a great deal of pride. I have no doubt that with the foundation that has been laid, the new Secretary-Treasurer will successfully carry on—provided he receives, as I have, the cordial, sympathetic support of referees and members of the Association. Publication of the next issue of *Methods of Analysis* is somewhat in doubt because of the uncertainty of the times, but it is not too soon to begin planning the work of revision, which should be ready for publication the latter part of 1945. I

shall, of course, as long as I am able to do so, maintain a very lively interest in all the affairs of the Association.

MR. SALE: As I stated in my announcement of this meeting, we certainly owe our heartfelt thanks for the service which Dr. Skinner has rendered over a long period of time. I think our appreciation of his services may take a more tangible form than a mere vote of thanks, but I shall now entertain a motion from the floor to the effect that we extend to him our heartfelt appreciation and thanks for the services given over a period of years.

MR. LEPPER: I move that the heartfelt thanks of the Association be extended to Dr. Skinner for his services as Secretary-Treasurer.

Seconded and carried.

DR. SKINNER: I accept that with a great deal of satisfaction.

#### SPECIAL MEETING OF BOARD OF DIRECTORS

At a special meeting of the Board of Directors of the Association, held at Washington, D. C., November 3, 1943, G. G. Frary presiding, and a quorum being present, it was resolved:

(1) That Henry A. Lepper, the Treasurer of the Association of Official Agricultural Chemists, be authorized and instructed to open a deposit account for and in the name of this organization with the Lincoln National Bank in the City of Washington, D. C., to deposit therein funds of the organization and that said account may be drawn on only by check signed in the name of this organization by its

*Secretary-Treasurer, Henry A. Lepper, or*

*Assistant Secretary-Treasurer, Marian E. Lapp*

(2) That the Secretary-Treasurer be authorized to provide a suitable seal for the use of the Association.

Both resolutions were seconded and carried.

#### REPORT OF AUDITING COMMITTEE

We, the Committee on Auditing, have examined the report and accounts of the Secretary-Treasurer, and find them balanced and in order. We have compared the Secretary-Treasurer's report with the report of the certified public accountant, Snyder, Farr & Company, which report is in agreement with the Secretary-Treasurer's accounts.

In the report of the certified public accountant we note the only assets to be reviewed are those listed under investments. An examination of the

bonds held in safe deposit at the Union Trust Company, Washington, D. C., was made, and it was found that they correspond with the listed assets, totaling a face value of \$35,000.

W. C. GEAGLEY  
M. S. ANDERSON

Approved.

## REPORT OF COMMITTEE ON NECROLOGY

The Committee on Necrology reports the passing of thirteen of our formers members; two in 1941, three in 1942, and eight in 1943. Included among these are three of the four persons who have held the directorship of the Office of State Cooperation in the U. S. Food and Drug Administration.

This office was established in 1914 and was first held by John S. Abbott. Prior to that time, he was for seven years the Food and Drug Commissioner of the State of Texas. He was head of the Office of Cooperation from 1914 to 1920. Since 1920 he had been associated with the margarine industry, and at the time of his death, January 23, 1943, he was Secretary and Research Director of the National Association of Margarine Manufacturers. He was 71 years of age. While he was the Chief of the Office of Cooperation, he invariably attended our meetings and was also an occasional visitor subsequent to relinquishing that office.

Mr. Abboott was succeeded in the Office of Cooperation by Theodore F. Pappe, who held the position for somewhat more than a year. He relinquished the office to return to his former work in the Bureau of Chemistry. At the time of this death, August 4, 1942, he was connected with the Buffalo Station of the U. S. Food and Drug Administration. Mr. Pappe had acted as Associate Referee on Pilocarpine in Tablets, on Small Quantities of Iodides in Mixtures, and on Mercurials.

Mr. Pappe was succeeded by Walter S. Frisbie, who in 1921 relinquished the office of Chief of the Bureau of Food, Drugs and Oil of the Department of Agriculture of the State of Nebraska. Mr. Frisbie passed away on February 19, 1942. He was one of our past presidents. An obituary has appeared in Volume 25, No. 1 of *The Journal*. Mr. Abbott outlived his two successors in that office.

In Volume 26 of *The Journal*, obituaries have appeared for G. S. Spencer, who died on December 16, 1942, and for Robert Harcourt, who died March 19, 1943.

Dr. Richard F. Jackson of the Bureau of Standards died on June 1, 1943, at the age of 62 years. An obituary has been prepared and will appear in Volume 26, No. 4 of *The Journal*.

Obituaries are being prepared for the following three persons: Dr. Samuel Palkin, Senior Chemist, Bureau of Chemistry and Soils, who

passed away May 2, 1943, at the age of 58; Dr. Fletcher P. Veitch, a man standing high in the branch of agricultural chemistry particularly pertaining to naval stores and leather, passed away October 15, 1943; Dr. Robert H. Kerr, Senior Chemist, Bureau of Animal Industry in charge of the Meat Inspection Laboratories, passed away on July 10, 1943. We all remember the very fine reports he has presented on meat and meat products.

Dr. Walter Beal Ellett, chemist of the Virginia Agricultural Experiment Station since 1906, died on May 12, 1943. He was born November 11, 1874, at Radford, Virginia; he obtained his B.S. degree in 1894, his M.S. degree in 1896 from the Virginia Polytechnic Institute, and his Ph.D. degree from Göttingen University, Germany, in 1904. He was an originator, with Prof. B. Tollens, of the Ellett-Tollens method for determining methylpentosans, which was reported on by the Referee on Foods and Feeding Stuffs at the 1906 and 1907 meetings of the Association. Ellett was a frequent attendant at the meetings of the Association between 1905 and 1925. He was chairman of the Committee on Nominations at the 1915 meeting, and wrote an obituary of Prof. R. J. Davidson, which was published in Vol. 3 of *The Journal* of the Association (p. 591).

Melvin Pingree of the American Agricultural Chemical Company and formerly of the Pennsylvania Agricultural Experiment Station died in June, 1943.

Frank L. Wollard of the Baltimore Station, Food and Drug Administration, died in May, 1941. He was an occasional visitor at our meetings.

George H. Adams, late Chief of the Boston Station of the U. S. Food and Drug Administration, died on December 31, 1941. He was an occasional visitor to our meetings. He was much respected both officially and personally by the Food and Drug Officials of the New England States, with whom he frequently came in contact.

H. C. LYTHGOE  
C. A. BROWNE

Approved.

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#### REPORT OF COMMITTEE ON NOMINATIONS

MR. SALE: In view of the prospective change in some of the fundamental offices of the Association, the President appointed by letter the members of this committee some weeks ago, and they have been in correspondence with each other and in deliberation here at this meeting. Dr. MacIntire will now report.

DR. MACINTIRE: This is a momentous meeting for our Association. As our President has said, several weeks ago he requested that Dr. White, Dr. Bailey, and I serve as a Committee on Nominations. We had considerable correspondence and have had several conferences since arriving in Washington. Your committee was strongly tempted to suggest an inno-

vation, if not a precedent. When we thought of your work, Mr. Sale, as President of this Association and the extra load placed upon you by Dr. Skinner's illness and absence, we had thought of suggesting the innovation of a "reelection." It was pointed out, however, that you had effectively and admirably reelected yourself, and anticipated our opportunity to establish an innovation.

We unanimously recommend that you elect as President for the ensuing year our present Vice-President, G. G. Frary of the State Chemical Laboratory of Vermillion, South Dakota; and as Vice-President, J. O. Clarke, Chief of the Central District, Food and Drug Administration, Chicago. Mr. President, I move you that this part of the Committee's report be adopted and that you elect these two gentlemen to the respective offices.

Seconded by W. W. Skinner, and carried.

DR. MACINTIRE: Mr. President, I move that you ask our distinguished friends, "Lord" Hermann C. Lythgoe and Dr. C. A. Browne, to escort our President-elect to the Chair.

At the conclusion of this ceremony Dr. Frary said: "Friends, I assure you that this is an honor that comes to me not without feelings of gratitude on my part toward my friends in this splendid organization of chemists. Nothing else could occur that could bring me satisfaction comparable to this. I feel my inadequacy and inability, perhaps my lack of professional deservedness of this honor. I assure you that I shall do my best to fill the position with credit to the Association. Again I thank you from the depth of my heart for the honor you have conferred upon me."

DR. MACINTIRE: The life of this Association might be considered as a parallel of the birth and development of a young man. In the incipient years we had a midwife, who was present at the birth of the Association and brought the child along until adolescent years. You all remember the devoted service of Dr. Harvey W. Wiley. Then for a period of ten years of adolescence and a little beyond, we had the fine services of Dr. Alsberg. For the last twenty-two years the work and hopes of those men have come into fruition under one whom we might consider the Guardian of this Association, the one who brought the "young man" into the full maturity of his stature and now grown to such proportions and wisdom that he probably does not require such guidance as has been given to him. I have not the temerity to try to express to you all the appreciation that I know each of us in his heart feels for the services rendered this Association by its Secretary during the last twenty-two years, for the man whom we have known, loved, and esteemed, and now that he is approaching his 70th year, whom we revere. Dr. Skinner made known his anticipation to retire in March 1944, and expressed the belief and conviction that he should relinquish the office of Secretary-Treasurer. He met with considerable opposition to the expression of his intention, but no amount of argument or

logic could budge him from his decision. We have not given up entirely, however, and your Committee is making a nomination and proposals for which we ask unanimous consent by a rising vote. Your Committee recommends that you elect Dr. W. W. Skinner as Secretary-Treasurer, *Emeritus* of the A.O.A.C. I move, Mr. President, that this action of the Committee be regarded as a unanimous expression of appreciation for one who has devoted himself to the interests of an organization that he has brought to its present splendid condition, such as is characterized by few similar organizations. (Several "seconds" were made and a rising vote was taken.)

And now, Mr. President, while I am on my feet—I read where Ruth said to Naomi, "Intreat me not to leave thee or to return from following after thee." We do not propose that *you*, Dr. Skinner, shall cease following after us! The Executive Committee has honored me, and I frankly wish it had not, for although I appreciate the distinction, I feel so definitely inadequate to give expression to the feeling that you all have, and I know I cannot give voice to those deep feelings which are in my own heart. I do not want to make this a funereal meeting, although each of us hates to see Dr. Skinner enter the list of those designated to retire. Nevertheless, we exult in the privilege he will have in playing with those grandchildren and imposing on them some of the discipline he has imposed on us.

The Executive Committee of this Association has taken an action, and that action was intended as a gesture—inadequate though it be—to indicate the appreciation that we as an Association feel for Dr. Skinner's long service. It is not in any way a compensation for what he has done, but it is a memento that he can keep in his home and point to with pride, if he will, and something that Mrs. Skinner will use and enjoy. The Executive Committee therefore empowered Miss Lapp to obtain a token that would be a concrete indication of the love that we feel for Dr. Skinner. Although Miss Lapp is most efficient and endowed with initiative, she is unable to place on this table the beautiful service of sterling silver that has been selected for Dr. Skinner. That service consists of a tea pot, a coffee pot, a hot water pot, a cream pitcher, and a sugar bowl, on a large sterling silver tray. In presenting this, Mr. President, by direction of your Executive Committee and in behalf of the membership of the Association, I should like to move that, when at hand, the silverware be presented to Dr. and Mrs. Skinner by the immediate past president of this Association.

DR. SKINNER: Mr. Chairman, this is very unexpected and very pleasing. I have not seen the silver service yet, and I think I shall reserve the right to thank you for it at the next meeting. I shall be prepared then to do so in a more gracious way. You have referred to my retirement that is pending. Part of the time after retirement I expect to spend with those

grandsons of mine, of whom Dr. MacIntire has spoken. I can only repeat what I have already said on another occasion:

I am not old—I cannot be old,  
Though threescore years and ten  
Have fritted away, like a tale that is told,  
The lives of other men.

I am looking forward to my retirement from Government service with a great deal of pleasure. I thank you for the compliments that come from you through Dr. MacIntire. I sincerely feel that it is for the good of the Association that I retire at this time. I am taking up a new line of work and hope to prove that life really begins at seventy.

DR. MACINTIRE: For the office of Secretary-Treasurer, your Committee has considered only one name, that of the man who has served loyally, faithfully, and effectively under the tutelage of our present Secretary-Treasurer. We nominate for the office of Secretary-Treasurer Henry A. Lepper and I move that the nomination be accorded the vote of affirmation. Seconded by J. W. Sale.

Mr. President, there are further nominations. Our retiring President, J. W. Sale, continues on the Executive Committee, and for additional members we nominate G. H. Marsh, W. H. Ross, and W. Catesby Jones.

A summary of the officers nominated follows:

*President:* G. G. Frary, State Chemical Laboratory, Vermillion, S. D.

*Vice-President:* J. O. Clarke, Food and Drug Administration, Chicago, Ill.

*Secretary-Treasurer Emeritus:* W. W. Skinner.

*Secretary-Treasurer:* Henry A. Lepper, Food and Drug Administration, Washington, D. C.

*Additional Members of Executive Committee:* G. H. Marsh, Montgomery, Ala.; W. H. Ross, Beltsville, Md.; W. Catesby Jones, Richmond, Va.; J. W. Sale, Washington, D. C.

A unanimous vote was cast for the officers nominated.

W. H. MACINTIRE

E. M. BAILEY

W. B. WHITE

Approved.

The silver service was presented to Dr. and Mrs. Skinner on December 8, 1943, in Dr. Skinner's office, J. W. Sale, President of the Association, for 1942-1943, making the presentation speech.

## REPORT OF COMMITTEE ON RESOLUTIONS

*Whereas*, the 58th annual meeting of the Association of Official Agricultural Chemists has been very successful notwithstanding the difficulties of holding a meeting during the war; and



*Whereas*, the various activities of the Association have been carried on under wise and sound leadership, it is particularly fitting at this time that we express our gratitude to those who have served as officers of the Association and to those who have contributed to the excellent program now concluded. Be it therefore

*Resolved*: That this Association express its appreciation to Dr. W. W. Skinner, who for 21 consecutive years as our Secretary-Treasurer and President has played a leading part in the progress of the Association. Upon his retirement we shall sadly miss his counsel and able guidance as an active member, but we hope to continue to enjoy his presence with us at our annual meetings in the years to come.

*Resolved*: That this Association extend its thanks to Mr. J. W. Sale, retiring president, who has served for 2 years, for his exceedingly interesting presidential address on "Some Highlights of War-time Food."

*Resolved*: That we extend our thanks to Mr. G. G. Frary as Vice-President for his able work and services, to Miss Marian E. Lapp, and her co-workers for their valuable assistance in making this meeting a success, and to our fellow members that have acted as sectional leaders, referees, associate referees, and collaborators.

*Resolved*: That through our Secretary we extend our thanks to the management of the Statler Hotel for the many courtesies extended to us.

H. H. HANSON

S. C. ROWE

Approved.

## CONTRIBUTED PAPERS

### VANILLIN FROM THE SHELLS OF TUNG NUTS\*

By MAX PHILLIPS (Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Washington, D. C.)

The fact that vanillin may be obtained from a lignin derivative was first observed in 1904 by Grafe.<sup>1</sup> He heated the dry residue of waste sulfite liquor with lime at 180°C. and isolated vanillin from the reaction product. No attempt was made to follow up this rather important observation until 1928, when Kürschner<sup>2</sup> began the publication of a series of papers describing the production of vanillin by heating waste sulfite liquor with sodium or potassium hydroxides. This reaction has since been extensively studied by other investigators<sup>3</sup> and several patents<sup>4</sup> have been granted on various improvements in the procedure. In 1940, Freudenberg, Lautsch, and Engler<sup>5</sup> observed that if an oxidizing agent, such as nitrobenzene, is added to the alkaline reaction mixture, substantial yields of vanillin may be obtained not only from waste sulfite liquor or ligninsulfonic acids, but also from isolated lignins, such as "cuproxam" or Willstätter lignins, and even directly from a lignin-containing substance such as spruce wood.

In connection with a study of the chemical composition of tung nut shells, the writer found that their lignin content, as determined by the fuming hydrochloric acid method,<sup>6</sup> was nearly 45 per cent, although the percentage of methoxyl in this lignin was considerably lower than that generally found in the lignins from wood. Therefore, in view of the relatively high percentage of lignin in tung nut shells, it was of interest to determine the yield of vanillin that might be obtained from this material. It was with this objective that the investigation recorded in this paper was undertaken.

### EXPERIMENTAL

The tung nut shells† were ground in a Wiley mill, and the following determinations were made.‡ All results were calculated on the moisture-free basis.

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\* Agricultural Chemical Research Division Contribution No. 121.

<sup>1</sup> *Monatsh.*, **25**, 997 (1904).

<sup>2</sup> *J. Prakt. Chem.*, [2] **118**, 238 (1928); Kürschner and Schramek, *Tech. Chem. Paper-Zellstoff-Fabr.*, **28**, 65 (1931); *Ibid.*, **29**, 35 (1932); Kürschner, *Ibid.*, **30**, 1 (1933).

<sup>3</sup> Hönig and Ruzicka, *Z. angew. Chem.*, **44**, 845 (1931); Shoruigin and Smolyaninova, *J. Gen. Chem. (U.S.S.R.)*, **4**, 1428 (1934); Tomlinson, Jr. and Hibbert, *J. Am. Chem. Soc.*, **58**, 345, 348 (1936); Hägglund and Bratt, *Svensk Papperstidn.*, **39**, 347 (1936).

<sup>4</sup> Sandborn, Salvesen, and Howard, *U. S. Patent* 2,057,117 (1936); Hibbert and Tomlinson, Jr., *U. S. Patent* 2,069,185 (1937); *British Patent* 465,708 (1937); Hatch, *U. S. Patent* 2,099,014 (1937); Sandborn, *U. S. Patent* 2,104,701 (1938).

<sup>5</sup> *Ber.*, **73**, 167 (1940).

<sup>6</sup> Goss and Phillips, *This Journal*, **19**, 341 (1936).

† The shells were kindly supplied by R. S. McKinney of the Tung Oil Laboratory of the Bureau of Agricultural and Industrial Chemistry, located at Gainesville, Fla.

‡ The analytical methods used were those described or referred to in a previous paper (Phillips, Goss, Davis, and Stevens, *J. Agr. Research*, **59**, 319 (1939)).

	<i>per cent</i>
Ash.....	2.73
Methoxyl.....	5.54
Alcohol-benzene extractives.....	12.14
Hot water extractives.....	5.19
1% hydrochloric acid extractives.....	13.94
Uronic acids (as anhydrides).....	4.68
Total furfural..... <sup>1</sup>	9.80
Lignin.....	44.80
Methoxyl in lignin.....	10.45

In order to determine the optimum temperature for obtaining the maximum yield of vanillin from tung nut shells, a series of experiments was carried out in which the temperature of the reaction was the sole variable. The experiments were conducted in an iron, high-pressure autoclave provided with a thermometer well and an electrically operated stirrer. The reaction chamber of the autoclave was placed in a metal bath (lead and tin) and heated by gas. The general procedure used was as follows:

#### EXPERIMENTAL PROCEDURE

##### PREPARATION OF VANILLIN

Fifty grams of ground tung nut shells (dried at 105°C.), 600 ml. of 8% aqueous NaOH solution, and 35 ml. of nitrobenzene were heated together in the autoclave. (It required 1.5–2 hours, depending upon when the mixture attained the desired temperature.) The heating was continued for 3 hours at the selected reaction temperature. After the reaction mixture had cooled to room temperature, it was removed from the autoclave and distilled in a current of steam until the aniline, nitrobenzene, and azobenzene were distilled over. After cooling, the residual mixture in the distilling flask was filtered, the insoluble material was washed with 5% aqueous NaOH solution, and the washings were added to the main filtrate. The residual material from this filtering and washing operation was discarded. The filtrate and washing solution were combined and then acidified with HCl, and the dark-brown precipitate was filtered off and extracted for 24 hours with benzene in a Soxhlet extractor. The filtrate was also extracted with benzene for 24 hours in a continuous extraction apparatus. The benzene solutions from both extraction operations were combined (total volume approximately 1 liter) and then extracted six successive times with a 5% NaHSO<sub>3</sub> solution, 150 ml. of the solution being used for each extraction. The NaHSO<sub>3</sub> solution was transferred to a 2 liter distilling flask, the solution was acidified with 40 ml. of concentrated H<sub>2</sub>SO<sub>4</sub>, and the flask and contents were partially evacuated with a water pump, while a small stream of air was passed through a capillary tube. When all the SO<sub>2</sub> had been expelled, the solution was transferred to a 1 liter volumetric flask, and the solution was diluted to the mark with distilled water.

The vanillin in this solution was determined by the Pritzker-Jungkunz' modification of the Hanuš<sup>2</sup> method, as follows:

##### DETERMINATION OF VANILLIN

Two 50 ml. samples were measured out with a pipet into 200 ml. Erlenmeyer flasks, and to each sample were added 15 grams of sodium acetate and 0.3 gram of

<sup>1</sup> *Chem. Ztg.*, **52**, 537 (1928).

<sup>2</sup> *Z. Untersuch. Nahr. Genussm.*, **10**, 585 (1905).

*m*-nitrobenzoylhydrazine in 15 ml. of hot water. The flasks were corked lightly and heated in a water bath at 60°C. for 0.5 hour. They were then allowed to stand at room temperature for 24 hours, after which time the precipitates were filtered into weighed Gooch crucibles, washed with cold water, and dried at 105°C. for 3 hours. The weight of the hydrazone precipitate multiplied by the factor 0.4825 gave the weight of vanillin in the sample.

The results obtained are given in Table 1, and it will be observed that the best yield of vanillin was obtained when the reaction temperature was 170°C.

TABLE 1.—*Yields of vanillin from tung nut shells*

(50 grams of ground tung nut shells, 600 ml. of 8% aqueous NaOH solution, and 35 ml. of nitrobenzene were heated together for 3 hours at various temperatures.)

REACTION TEMP.	WEIGHT	CALC'D ON BASIS OF TUNG NUT SHELLS	CALC'D ON BASIS OF LIGNIN IN TUNG NUT SHELLS
°C.	grams	per cent	per cent
150	1.2738	2.54	5.07
160	1.4820	2.96	5.90
170	1.6100	3.22	6.40
180	1.3120	2.62	5.21

Experiments were also conducted in which the concentration of the alkali used in the same reaction was varied, but no significant changes in the yield were obtained.

Following the procedure described for the production of vanillin from tung nut shells and conducting the reaction at 170°C., the writer also carried out an experiment for the purpose of actually isolating vanillin. The procedure used follows:

#### ISOLATION OF VANILLIN

After the bisulfite addition compound had been decomposed, the solution was extracted with ether, and the ether solution was dried over anhydrous sodium sulfate. After removal of the ether by distillation, an oil was obtained that solidified on standing. After several recrystallizations from hot ligroin and decolorization with norit, colorless crystals were obtained. These crystals were identified as vanillin by their melting point. The semicarbazone of this crystalline substance was also synthesized, and it was identified by its melting point as vanillin semicarbazone. The yield of vanillin was approximately 1.5 per cent of the weight of the tung shells.

#### SUMMARY

A study was made of the preparation of vanillin from tung nut shells. The maximum yield of vanillin, as determined analytically, amounted to 3.2 and 6.4 per cent of the tung nut shells and of the lignin in the shells, respectively.

## MODIFIED KREIS TEST APPLICABLE TO COSMETIC PREPARATIONS\*

By J. H. Jones (Cosmetic Division, U. S. Food and Drug Administration, Washington, D. C.)

The Kreis test<sup>1,2</sup> is widely used as a means of detecting deterioration in fats and oils. Numerous modifications of this test are available, but no study appears to have been made of its application to cosmetics, and none of the published methods seems entirely suitable for this purpose.

It has been shown<sup>3,4</sup> that epinhydrin aldehyde is responsible for the production of the pink color in this test. This aldehyde is probably present in the deteriorated fat as an acetal from which it is liberated on treatment with concentrated hydrochloric acid. Taufel and his co-workers<sup>4,5,6</sup> found that the epinhydrin aldehyde could be aerated from acidified mixtures and absorbed in a mixture of equal volumes of concentrated hydrochloric acid and 0.1 per cent phloroglucinol solution to develop the characteristic color. They state that this modification gives an approximately quantitative determination of the epinhydrin aldehyde present.

This procedure may be satisfactory for colored non-perfumed cosmetics, but many perfumes, flavors, and essential oils are volatilized with the epinhydrin aldehyde and give colored solutions or precipitates with the absorption reagent. Taufel<sup>5</sup> suggested the elimination of volatile aldehydes by steam distillation since epinhydrin aldehyde is not volatilized unless the mixture is acidified. Attempts to remove perfumes and flavors from cosmetic creams in this manner were not successful. Excessive foaming usually resulted, and it was very difficult to remove the last traces of the interfering substances.

The experiments made by the writer show that although concentrated acid is necessary to liberate the epinhydrin aldehyde, the absorption can be made in a dilute acid solution without appreciable loss of sensitivity. When this dilute acid absorption solution is used most perfumes, flavors and essential oils do not seem to interfere.

Many cosmetic preparations are solids, or contain substances that solidify on treatment with hydrochloric acid, and cannot be tested directly. Ether, benzene, and kerosene have been used as solvents for oils and fats to which the Kreis test was to be applied. Neither of these was found entirely satisfactory for cosmetic products. Experiments showed, however, that mineral oil dissolves, or at least prevents the precipitation of

\* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 27, 28, 1943.

<sup>1</sup> Kreis, *Chem. Z.*, **26**, 897 (1902).

<sup>2</sup> Kerr, *Ind. Eng. Chem.*, **10**, 471 (1918).

<sup>3</sup> Powick, *J. Agr. Research*, **26**, 323 (1923).

<sup>4</sup> Taufel and Russow, *Z. Untersuch. Lebensmitt.*, **65**, 540 (1933).

<sup>5</sup> Taufel, *Chem. Umschau. Fette*, **39**, 147 (1932).

<sup>6</sup> Taufel and Sadler, *Z. Anal. Chem.*, **90**, 20 (1932).

paraffin, beeswax, etc., which are so frequently present in cosmetic emulsions.

The following method is tentatively suggested for the detection of epinhydrin aldehyde in cosmetic products containing perfumes, flavors, and essential oils.

#### PROPOSED METHOD

##### APPARATUS

A convenient aeration apparatus for use with 2–10 gram samples may be constructed from three 1 × 6 inch test tubes. Fit each test tube with a two-holed rubber stopper, each stopper carrying one short piece of glass tubing and one long enough to reach to the bottom of the test tube. Connect in series with rubber tubing so that when attached to the air line the air enters each test tube through the long piece of glass tubing. Tube 1 serves as the aerator, Tube 2 is loosely packed with glass wool to filter any entrained oil, and Tube 3 is the absorber.

##### REAGENTS

(a) *Absorption solution*.—Dissolve 0.2 gram of phloroglucinol in 100 ml. of (1+9) HCl. Prepare a fresh solution daily.

(b) *Hydrochloric acid*.—37%. Use acid that gives no color with an equal volume of the absorption solution.

(c) *Mineral oil*.—U.S.P. light mineral oil, or a similar grade.

##### PREPARATION OF SAMPLE

(1) *Liquid samples consisting essentially of oils*.—Weigh 2–10 grams of the sample directly into the aeration tube.

(2) *Solids or emulsions containing at least 50% oil*.—Mix a weighed sample of 2–10 grams in the aeration tube with about two volumes of mineral oil. Heat in a boiling water bath until the sample liquefies, mix thoroughly, and cool to room temperature.

(3) *Emulsions or solutions containing a low percentage of oil*.—Evaporate a weighed sample containing 2–10 grams of oil in a vacuum desiccator at room temperature until the oil content is over 50%. Mix with mineral oil in the aeration tube as directed in (2).

##### DETECTION OF EPINHYDRIN ALDEHYDE

Place the absorption solution in the absorber, using for routine tests 2 ml. for each gram of oil in the sample. Add to the aeration tube containing the prepared sample a volume of concentrated HCl equal to the total volume of sample and mineral oil. Assemble the apparatus and pass air through it at a rapid rate for 30 minutes. A pink color in the absorption solution indicates deteriorated oils. A yellow color or cloudiness should be disregarded.

##### COMMENTS

The procedure described obviously eliminates the interference due to the color of the product. To check the effect of other aldehydes, perfumes, flavors, and essential oils about 0.2 gram of various substances of this type was mixed with 5 ml. of mineral oil and tested. The substances listed in Table 1 gave no color, although in a few cases the absorption solution became cloudy. With Taufel's absorption solution (containing concentrated hydrochloric acid) many of these substances produced colors that varied from yellow to deep red.

TABLE 1.—*Substances that do not give positive tests*

Anisic Aldehyde	Phenylacetaldehyde
Anethol	Phenylethyl alcohol
Benzaldehyde	Terpineol
Camphor	Vanillin
Coumarin	Oil of bay
Ethyl vanillin	Oil of bergamot
Eucalyptol	Oil of cloves
Geraniol	Oil of citronella
<i>n</i> -Heptaldehyde	Oil of lemon
Heliotropin	Oil of orange
$\beta$ -Ionone	Oil of patchouli
Iso-eugenol	Oil of peppermint
Iso-safrole	Oil of pine
Menthol	

Cinnamic aldehyde, eugenol, oil of cinnamon, and oil of cassia gave a yellow color with both absorption reagents. The color, however, was a pure yellow and would not be mistaken for a positive test.

Attempts to use the proposed procedure for the quantitative estimation of epinhydrin aldehyde were not successful. However, it can be used to classify the products tested. If 2 ml. of absorption solution is used for each gram of oil in the sample fresh oils give no coloration, oils which are slightly deteriorated produce a light pink color, and badly decomposed oils give a deep pink color.

Samples of lipstick and cold cream were prepared from slightly deteriorated oils by standard procedures. The samples when tested gave colors of approximately the same intensity as the original oil. These tests and other similar ones indicate that the moderate temperatures, i.e., 60°–80°C., ordinarily used in the manufacture of cosmetic products of this type, have little effect upon the epinhydrin aldehyde.

Two samples of a sun-tan oil, collected at the same time, were available in the laboratory. This product contained about 4 per cent of heliotropin and a small amount of color dissolved in a mixture of oils. After about one-half of the contents had been removed from one of the bottles, both bottles were stored in the same place for several months. When examined, the partly filled bottle had an "off" odor, but owing to the presence of the perfume this odor could not definitely be classed as "rancid." No odor, other than that of the perfume, could be detected in the other sample. When tested by the proposed procedure the partly filled bottle gave a strongly positive, and the full bottle a negative test.

#### SUMMARY

A modified Kreis test applicable to cosmetic products has been presented. An aeration procedure is used with a modified absorption reagent, which eliminates the interference of many other volatile substances.

Directions for testing various types of cosmetics are given.

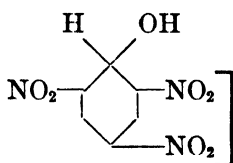
## SEPARATION AND DETERMINATION OF 2,4-DINITROANILINE IN D&C ORANGE NO 17\*

By O. L. EVENSON (Cosmetic Division, U. S. Food and Drug  
Administration, Federal Security Agency,  
Washington, D. C.)

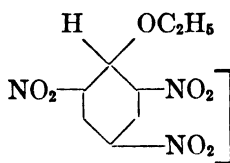
D&C Orange No. 17 (also known as Permanent Orange) 1- (2,4-dinitrophenylazo)-2-naphthol, is one of the colors certifiable by the Food and Drug Administration for coloring drugs and cosmetics.<sup>1</sup> The free intermediate, 2,4-dinitroaniline, is permitted in a certified batch of this color to the extent of not more than 0.2 per cent.

The colorimetric method outlined here for the quantitative determination of 2,4-dinitroaniline is based upon the measurement of the color produced when sodium hydroxide is added to an alcoholic solution of the intermediate.

Many polynitro compounds form colored derivatives when treated with alkali. Meyer and Michler<sup>2</sup> first investigated this. They added sodium amalgam to dinitro benzoic acid dissolved in sodium hydroxide and obtained a red color. Subsequently a number of other investigators reported analogous results with other polynitro compounds. The reaction has been used analytically for the determination of benzene in air<sup>3</sup> and trinitro benzene in dinitrobenzene.<sup>4</sup> The test for thiophen in benzene is also based upon this reaction. According to Hantzsch and Picton<sup>5</sup> the colored compound produced by the action of sodium hydroxide upon an alcoholic solution of trinitro benzene is a derivative of nitronic acid.



Nitronic Acid



Colored Compound

Alcohol is not in all cases necessary for the formation of color; 2,4-dinitroaniline forms a red color in the presence of sodium hydroxide alone. This points to salt formation, involving two nitro groups, since mononitro compounds do not react in this manner.

\* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 27, 28, 1943.

<sup>1</sup> S.R.A., Food, Drug, and Cosmetic No. 3.

<sup>2</sup> V. Meyer and W. Michler, *Ber.*, 6, 746-8 (1874).

<sup>3</sup> B. H. Dolin., *Ind. Eng. Chem., Anal. Ed.*, 15, 242-7 (1943)

<sup>4</sup> M. L. Moss and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.*, 14, 861-2 (1942).

<sup>5</sup> A. Hantzsch and N. Picton, *Ber.*, 42, 2119-28 (1909).



## METHOD

## REAGENTS

*Sodium hydroxide solution.*—Dissolve 30 grams of carbonate-free NaOH in CO<sub>2</sub>-free water and dilute to 100 ml. Protect the solution with a soda-lime tube. Or prepare a CO<sub>2</sub>-free solution of the same strength from CO<sub>2</sub>-free water and (1+1) NaOH from which the Na<sub>2</sub>CO<sub>3</sub> has been permitted to settle out.

*Ethyl alcohol.*—95%.

*Standard solution of 2,4-dinitroaniline.*—Dissolve 50 mg. in 500 ml. of 95% ethyl alcohol.

## DETERMINATION

Dissolve 0.1 gram of color in about 3 ml. of concentrated H<sub>2</sub>SO<sub>4</sub> in a tall 300 ml. beaker. Add *carefully* small quantities of alcohol, stirring after each addition until about 30 ml. has been added. Then add 50 ml. of water and mix. Evaporate on a steam bath until about 30 ml. remains, using a gentle air blast to hasten the process. Transfer the contents to a 100 ml. volumetric flask, cool, and dilute to the mark with water. Mix, and filter on a small filter paper. With a pipet transfer a 50 ml. aliquot of

TABLE 1.—*Recovery of 2,4-dinitroaniline from D&C Orange No. 17*

NO.	WGT. COLOR	2,4-DINITROANILINE		RECOVERY
		ADDED	FOUND	
	mg.	mg.	mg.	per cent
1	100	2.5	2.37	94.8
2	50	2.5	2.37	94.8
3	25	0.1	0.097	97.0
4	100	0.5	0.475	95.0
5	50	0.5	0.475	95.0
6	50	1.0	0.95	95.0
7	100	3.0	2.85	95.0
8	100	4.0	3.80	95.0
9	100	5.0	4.80	96.0
10	50	2.5	2.45	98.0
11	100	0.5	0.47	94.0
12	100	1.0	0.94	94.0
			Average	95.3

the filtrate to a 250 ml. separatory funnel. (If the sample contains more than 0.2% of intermediate, use a smaller aliquot and dilute to 50 ml. with water.) Make slightly alkaline with the NaOH solution and extract the intermediate with three portions (50–40–40 ml.) of ether. Wash the combined ether extracts with three 10 ml. portions of water. Drain the wash water carefully, after allowing ample time for the layers to separate. Transfer the solvent to a beaker and wash the funnel with ether, adding this to the main extract. Let the solvent evaporate in a warm place. Dissolve the residue in warm alcohol and transfer (with alcohol) to a 50 ml. volumetric flask. Cool, add alcohol to the mark, and mix. Transfer an appropriate aliquot to a 25 ml. volumetric flask, and dilute to 20 ml. with alcohol. (If the quantity of intermediate in the original aliquot used for the ethereal extraction is less than 0.05 mg., the 50 ml. flask may be dispensed with. In this case transfer the alcoholic solution of the ethe-

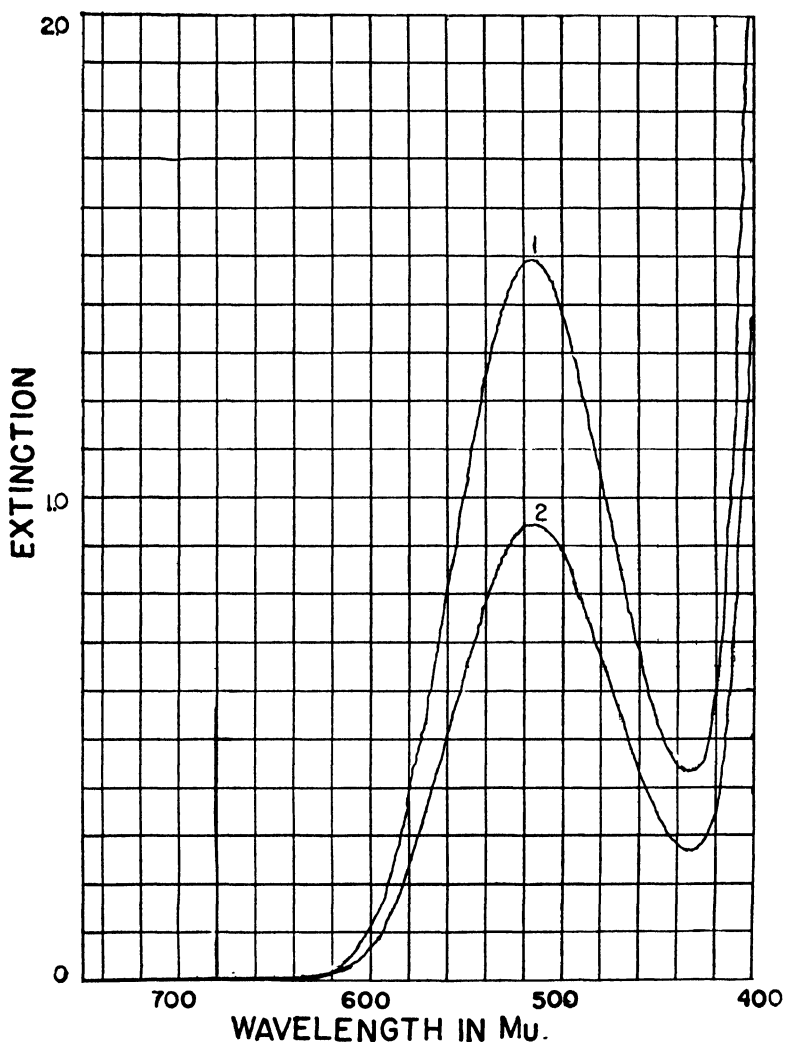


FIG. 1.—SHOWING EXTINCTION CURVES OF 2,4-DINITROANILINE, 0.8 MG. FOR NO. 1 AND 0.5 MG. FOR NO. 2, DISSOLVED IN 20 ML. OF ALCOHOL PLUS 5 ML. OF SODIUM HYDROXIDE.

real residue directly into the 25 ml. volumetric flask and dilute to 20 ml. with alcohol.) Prepare standards in 25 ml. volumetric flasks and dilute to 20 ml. with alcohol. Add 5 ml. of the NaOH solution to the standards and the unknown, mix, and pour the contents into suitable test tubes. Compare the unknown with the standards.

Results obtained with the method are shown in Table 1. A sample of D&C Orange No. 17 was purified by repeated extraction with benzene until no test for the intermediate could be obtained. Known quantities of intermediate in alcoholic solution were then added to weighed aliquots

of the purified color. The alcohol was evaporated on the steam bath and recoveries determined. The results show that a recovery of approximately 95 per cent may be expected.

Spectrophotometric extinction curves for the red color produced in this method are shown in Figure 1.\* These were obtained with a recording spectrophotometer. The absorption data are recorded as extinction (negative log of transmittancy). The maxima lie between 510 and 520  $m\mu$ . The intermediate, 0.8 mg. for curve No. 1 and 0.5 mg. for curve No. 2, was dissolved in 20 ml. of alcohol plus 5 ml. of sodium hydroxide solution.

The volume of sodium hydroxide and alcohol specified in the method was found to be the optimum for sensitivity and convenience of manipulation. The color so obtained remains stable for about one hour. In a volume of 25 ml., 0.005 mg. of the intermediate or a concentration of one part in five million, will give a distinct pink coloration.

#### SUMMARY

A colorimetric method has been presented for the quantitative determination of 2,4-dinitroaniline in D&C Orange No. 17. Recoveries of 95 per cent are indicated. The intermediate is removed from the color and quantitatively estimated by the depth of color it produces with aqueous sodium hydroxide in alcoholic solution. The spectral characteristics of this color are shown by extinction curves.

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#### ASSAY, PURIFICATION, AND SPECTRUM OF D&C RED NO. 18†

By S. H. NEWBURGER (Cosmetic Division, U. S. Food and Drug Administration, Washington, D. C.)

An investigation was undertaken to determine whether D&C Red No. 18 could be sulfonated to a water-soluble product that would lend itself readily to the customary assay of reduction with titanium trichloride. The following procedure was found to give reproducible analytical results:

Weigh 0.25 gram of the powdered dye into a 50 ml. beaker, add 6 ml. of 15% fuming  $H_2SO_4$ , cover with a watch-glass, and allow to react at room temperature for 30 minutes, with an occasional swirling of the flask. Pour the sulfonated product *cautiously* through a funnel into a 250 ml. volumetric flask containing about 125 ml. of cold water; cool, dilute to the mark with water, and mix. Pipet a 100 ml. aliquot into a 500 ml. titration flask, dilute to 200 ml. with water, add 30 grams of sodium bitartrate, heat to boiling, and titrate with 0.1  $N$   $TiCl_3$  in an atmosphere of  $CO_2$ . The end point is a sharp change from pale orange to almost colorless. 1 gram of D&C Red No. 18 = 195.85 ml. of 0.1  $N$   $TiCl_3$ .

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\* The writer is indebted to S. H. Newburger of this Division for the spectrophotometric curves shown here.

† Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 27, 28, 1943.

## EFFECT OF TIME AND TEMPERATURE

Two samples of D&C Red No. 18 were sulfonated for varying time intervals at room temperature and also at steam-bath temperature. The effect of the fuming sulfuric acid on the assay value was noted. The results are presented in Table 1.

It is apparent from Table 1 that a variation in the time of sulfonation at room temperature from 15 to 120 minutes does not alter the result of the analysis. However, if the sulfonation is carried out at the temperature of the steam bath, there is a lowering of the assay value with increased time of sulfonation. After two hours the decrease was about 5 per cent. These data indicate that sulfonation at room temperature with 15 per cent fuming sulfuric acid does not destroy any of the dye.

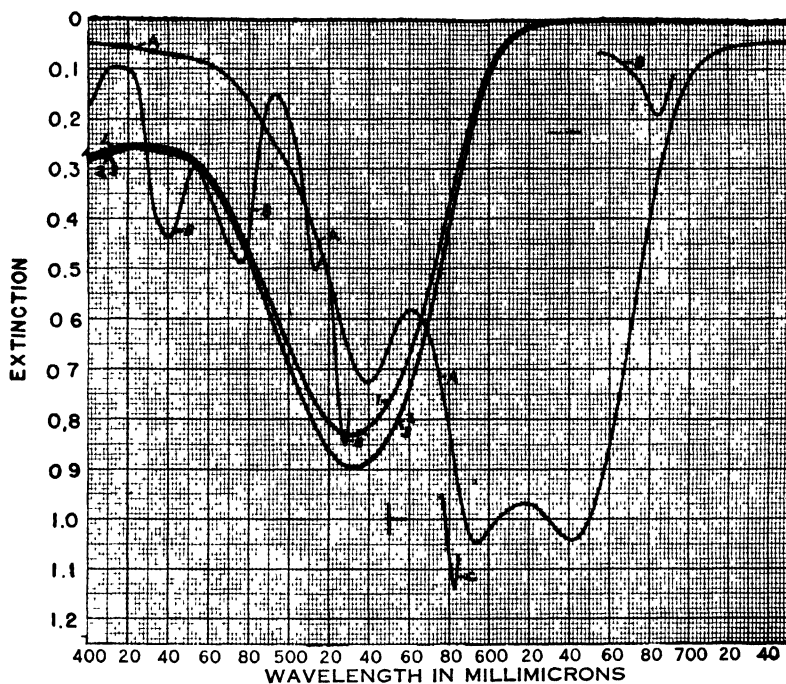


FIG. 1.—EXTINCTION CURVES OF D&C RED NO. 18 AT VARIOUS STAGES OF PURIFICATION.

- 1 = Commercial Sample of D&C Red No. 18
- 2 = 1st Purification of Commercial Sample
- 3 = 2nd Purification of Commercial Sample
- Concentration—10 mg./liter
- Solvent—chloroform
- Cells—1 cm.

A = Signal Lunar White Glass H-6946236

B = Corning Didymium Glass 512, 6.0 mm.

C = Corning Didymium Glass 592, 4.02 mm.

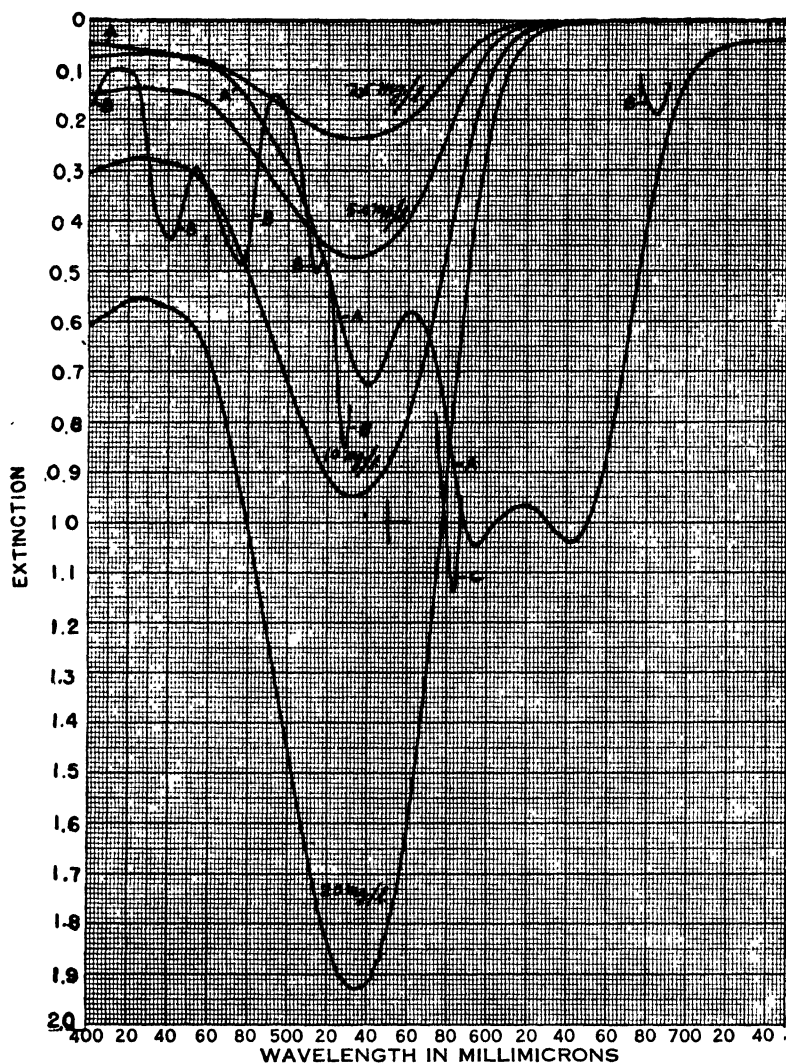


FIG. 2.—EXTINCTION CURVES OF D&C RED NO. 18.

Dye concentrations calculated on basis of pure dye content of D&C Red No. 18

Solvent—Chloroform

Cells—1 cm.

A = Signal Lunar White Glass H-6946236

B = Corning Didymium Glass 512, 6.0 mm.

C = Corning Didymium Glass 592, 4.02 mm.

The commercial sample of D&C Red No. 18 contained 91.2–91.5 per cent of pure dye and 0.6 per cent of matter, volatile at 135°C. The color had a strong odor and a dusky reddish brown hue, and did not melt

TABLE 1.—*Assay of D&C Red No. 18*

SAMPLE NO.	TEMPERATURE	TIME OF SULFONATION	DYE
	°C.	minutes	per cent
A*	28	30	91.2
A	28	60	91.2-91.5
A	28	120	91.2-91.5
B†	26.5	15	95.4-95.7
B	26.5	30	95.7
B	26.5	60	95.7
B	26.5	120	95.4-96.0
B	Steam bath	30	94.6-94.9
B	Steam bath	120	90.2-90.7

\* Commercial preparation.

† Sample A after purification.

sharply. Complete melting was observed at 214°C. Since D&C Red No. 18 has a low solubility in alcohol, whereas the intermediates are soluble, a purification was carried out as follows:

Dissolve about 20 grams of D&C Red No. 18 in 150 ml. of  $\text{CHCl}_3$  by heating on the steam bath. Add 1000 ml. of alcohol and continue to heat on the steam bath for 90 minutes. Cool, centrifuge, and pour off the liquid. Add more alcohol, stir thoroughly, and again centrifuge. Transfer the insoluble material to a Buchner funnel with the aid of water, drain with suction, and dry overnight at 135°C.

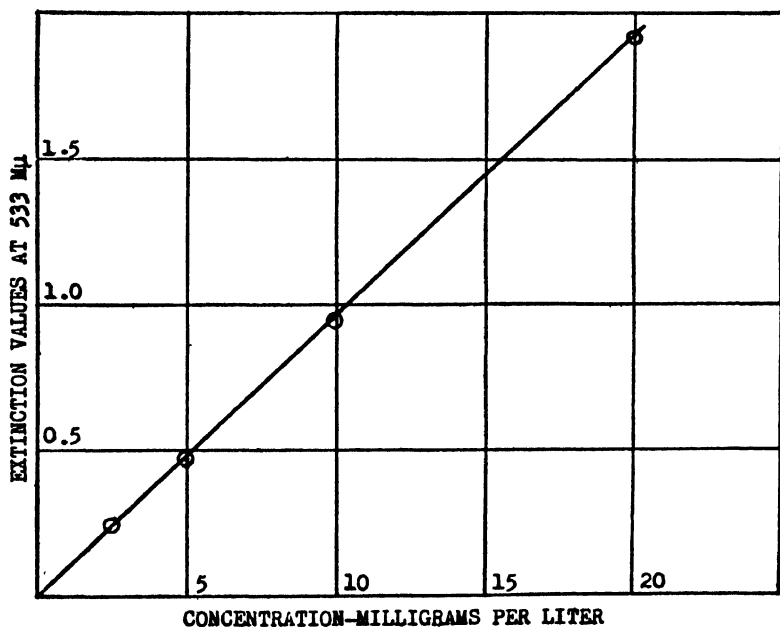


FIG. 3.—CONCENTRATION VERSUS EXTINCTION VALUES.

The dye recovery was 16.5 grams (82.5 per cent), and the material assayed 95.4–95.7 per cent.

No further increase in purity was obtained by a second purification. The dye content was 95.7 per cent with 0.1 per cent of volatile matter (at 135°C.). The dye had a deep reddish hue and was odorless, but still did not melt sharply. Complete melting was noted at 223°C.

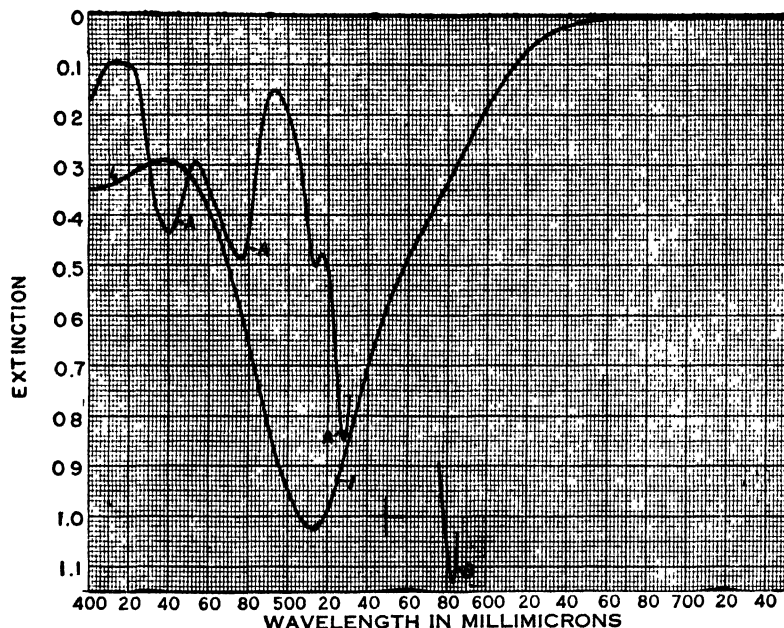


FIG. 4.—EXTINCTION CURVE OF D&C RED NO. 18 SULFONATED FOR 30 MINUTES AT 32°C.

1 = Sulfonated D&C Red No. 18

Concentration—10 mg./liter on basis of pure dye content of unsulfonated D&C Red No. 18

Solvent—dilute sulfuric acid (pH-2.1)

Cell—1 cm.

A = Corning Didymium Glass 512, 6.0 mm.

B = Corning Didymium Glass 592, 4.02 mm.

The effect of the drying at 135°C. on the chemical stability of D&C Red No. 18 was also ascertained. Two samples, one of which was dried for 6 hours and the other for 23 hours, gave identical assays, indicating that at 135°C. there is no progressive deterioration with increased exposure to heat.

#### SPECTROPHOTOMETRIC DATA

The various stages of purification were examined spectrophotometrically, and it is apparent from Figure 1 that the singly and doubly purified

products are of the same purity. Assuming the validity of Beer's law and using the dye content of 95.7 per cent for the purified material, the writer estimates that the dye content of the commercial sample is 88.7 per cent. By sulfonation a value of 91.2–91.5 per cent was obtained. The discrepancy may be due to reducible non-dye impurities.

Extinction curves of D&C Red No. 18 in chloroform solutions of varying concentrations were investigated in the spectral region 400–750  $m\mu$  with a General Electric recording spectrophotometer having an 8 millimicron slit (Figure 2). The color has an absorption peak at 532.5 ( $\pm 2$ )  $m\mu$ . The extinction ratio 513  $m\mu$ /554  $m\mu$  is 1.0 ( $\pm 0.02$ ), and it can be used as an additional aid in the identification of the dye. Extinction values at the absorption peak plotted against concentration indicate that Beer's law is valid (Figure 3). Hence quantitative spectrophotometric determinations are possible.

NOTE: The observed wave lengths were corrected to within  $\pm 2.0$   $m\mu$  of their true values with the aid of didymium glasses tested by the National Bureau of Standards.

Figure 4 shows the spectrophotometric curve of sulfonated D&C Red No. 18. The hue of the sulfonated color incidentally varies with the pH of the solution.

#### SUMMARY

A method has been developed for the assay of D&C Red No. 18.

A purification procedure for this color has been outlined.

Extinction curves of D&C Red No. 18 have been investigated in the spectral region 400–750  $m\mu$ . These data can be used for the identification and quantitative estimation of the dye.

The extinction curve of sulfonated D&C Red No. 18 has also been obtained in the spectral region of 400–750  $m\mu$ .

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#### CONDITIONS FOR COMPLETE ACID INVERSION IN ANALYSIS OF FINAL CANE MOLASSES

By F. W. ZERBAN, J. E. MULL, and JAMES MARTIN (New York  
Sugar Trade Laboratory, New York, N. Y.)

The quantity of acid, and the temperature and time required for complete inversion which have been established experimentally for pure sucrose are used quite generally without material change for sucrose determinations in final cane molasses although this practice has been questioned from time to time. H. S. Walker<sup>1</sup> recommends the addition of 1–2 ml. of hydrochloric acid (1+1), in excess of the 10 ml. used for inversion, to neutralize the alkalinity produced by clarification with an

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<sup>1</sup> *Hawaiian Planters' Record*, 15, 296 (1916).



excess of basic lead acetate. But even if this excess is previously removed by means of potassium oxalate or other deleading agent, a molasses solution highly buffered by salts of weak organic acids and other non-sugars will have a smaller acidity than an unbuffered solution of pure sucrose when the same quantity of acid is added to both. This buffer effect of molasses solutions is overcome in Saillard's method of neutral double polarization<sup>2</sup> by adding an amount of hydrochloric acid equivalent to the salts in the molasses solution, in excess of the quantity usually employed for the inversion of pure sucrose. Saillard's method is not practical for routine analyses because the Clerget divisor must be determined for each individual sample.

The method prescribed by the U. S. Treasury Department for the determination of sucrose in molasses, which is essentially Jackson and Gillis method No. IV,<sup>3</sup> specifies inversion at 60° for 10 minutes. This is well within the range of the 8–11 minutes within which these authors observed no perceptible change in the invert reading of completely inverted pure sucrose.

The possibility of incomplete inversion in the analysis of final cane molasses under these conditions is recognized by the following statement in Circular C 440 of the National Bureau of Standards, p. 131: "It is true that at 60° many final molasses are not completely inverted in the specified period of time. Such samples would require either a prolonged time of inversion at 60° or an elevation of the temperature to 70°, and the value of the divisor under these altered conditions requires determination." In the paragraph immediately following, inversion at 70°, 60°, and at room temperature, alternatively, is recommended. More specifically, Jackson and Gillis advocate for inversion at room temperature 30.8 hours at 20°, 14.6 hours at 25°, or 7.1 hours at 30°. The A.O.A.C. methods<sup>4</sup> prescribe 24 hours when the temperature is not below 20°, and 10 hours when it is above 25°.

The present investigation was undertaken to ascertain under what conditions an average blackstrap molasses is completely inverted. Because of the widely varying composition of blackstraps these conditions may be expected to vary from case to case. To solve the problem completely it would be necessary to remove all the sucrose from the molasses without changing its composition in any other respect, then to add known quantities of pure sucrose and determine it. This would be a very formidable experimental task and would have to be repeated with a number of samples in order to arrive at average values. The problem is further complicated by the presence of reversion products that are also hydrolyzed by the acid. This usually causes a plus error in the sucrose determined by any of the

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<sup>2</sup> *Proc. 8th Intern. Congr. Appl. Chem.*, 25, 541 (1913).

<sup>3</sup> *Bur. Standards Sci. Paper* 375 (1920).

<sup>4</sup> *Methods of Analysis*, A.O.A.C., 1940, 495.

acid hydrolysis methods, as shown by Zerban and Gamble.<sup>5</sup> But as long as acid hydrolysis methods are used this error cannot be avoided. The simplest way to determine the proper conditions for complete inversion is to use as the criterion the maximum levorotation (minimum dextrorotation), as was done by Jackson and Gillis. When the levorotation is plotted against time at any given temperature of inversion, the curve gradually rises to a maximum, owing to inversion, and then descends, owing to destruction of invert sugar. The top of the curve indicates maximum inversion and minimum destruction.

Another question arises when the percentage of sucrose is to be calculated. Until the correct Clerget divisors for blackstraps can be determined by the method outlined above it will be best to use the divisors established for sucrose, as is generally done with the invertase method. In the case of that method it has been experimentally established that the divisor found for sucrose is correct for impure products also.<sup>6</sup> Jackson and Gillis give for pure sucrose determined by Method No. IV the basic divisor 132.63, no matter what temperature, up to 60°, is used for the inversion, and they concluded that under these conditions there is no destruction of invert sugar during the time required for complete inversion. But later Jackson and MacDonald<sup>7</sup> found that the basic divisor decreases with an increase in the temperature of inversion, from 132.66 at room temperature to 132.56 at 60°. In the tables the sucrose has been calculated on the basis of both the Jackson and Gillis and the Jackson and McDonald divisors, and it will be noted that the differences between the values calculated in these two ways have no practical significance.

Three series of experiments with Cuban and Puerto Rican final molasses were made to ascertain the time necessary to attain maximum levorotation at various temperatures. In the first series 14 samples were inverted for 8, 10, and 12 minutes at both 60° and 70°, and for comparison at 28° for 24 hours. In each analysis half-normal solutions of the molasses were prepared, and the final readings were taken in quarter-normal solution at 20.0°. All analyses were made in duplicate by two different chemists, and the averages were taken. The results of this series are shown in Table 1.

The maximum average invert reading was established after inverting at 28° for 24 hours. Almost the same reading was obtained after heating to 70° for 8, 10, or 12 minutes; the slight falling off upon heating for more than 8 minutes is not significant. The results clearly show that heating for 10 minutes at 60° is insufficient for complete inversion, as the reading still increased nearly 0.2 in the next two minutes.

At the bottom of the table the average sucrose figures are also given, calculated by the basic divisors of Jackson and Gillis (J. & G.) and also

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<sup>5</sup> *Ind. Eng. Chem., Anal. Ed.*, 5, 34 (1933).

<sup>6</sup> *This Journal*, 11, 167 (1928).

<sup>7</sup> *Ibid.*, 22, 580 (1939); Natl. Bur. Standards Circ. C 440, 155 (1942).

by those of Jackson and McDonald (J. & McD.), valid in each case for method No. IV at the temperature employed for the inversion. The new concentration factor of Jackson and McDonald, 0.0794, was used to correct the basic divisor to the concentration of solids in the quarter-normal weight solution.

Owing to the fact that the basic divisor for inversion at 70° is smaller than that for inversion at 60° or below, the sucrose results obtained at this

TABLE 1.—*Effect of temperature and time on acid inversion (Series 1)*

NO.	INVERT READINGS						
	8 MIN.	60° 10 MIN.	12 MIN.	8 MIN.	70° 10 MIN.	12 MIN.	28° 24 HR.
1	-16.20	-17.80	-17.80	-18.20	-18.00	-18.00	-18.20
2	-15.80	-17.00	-17.20	-17.20	-17.40	-17.40	-17.40
3	-19.00	-19.60	-19.40	-19.60	-19.60	-19.60	-19.20
4	-18.60	-19.80	-19.80	-19.80	-19.80	-19.60	-20.10
5	-18.20	-19.40	-19.40	-19.40	-19.40	-19.40	-19.40
6	-17.80	-19.00	-19.00	-19.40	-19.20	-19.20	-19.40
7	-16.00	-17.80	-17.80	-18.00	-17.80	-18.00	-18.00
8	-17.60	-18.40	-18.60	-18.80	-18.80	-18.60	-18.80
9	-16.00	-17.00	-17.40	-17.60	-17.60	-17.60	-17.60
10	-18.00	-18.60	-19.00	-18.80	-18.80	-19.00	-18.60
11	-15.60	-17.40	-17.60	-17.80	-17.80	-17.80	-17.80
12	-15.40	-16.00	-16.40	-16.80	-16.80	-16.80	-16.80
13	-15.80	-17.20	-17.60	-17.80	-17.80	-17.80	-18.00
14	-15.00	-15.80	-16.00	-16.00	-16.00	-15.80	-16.00
Av.	-16.79	-17.91	-18.07	-18.23	-18.20	-18.19	-18.24

Average Sucrose (per cent)							
J. & G.	36.58	37.43	37.55	37.72	37.69	37.69	37.68
J. & McD.	36.60	37.45	37.57	37.74	37.72	37.71	37.67

temperature are a little higher than those found for inversion at room temperature, even though the invert readings were a little lower, but the differences are slight, amounting to 0.01–0.07. The important result of this series is that the official procedure of heating to 60° for 10 minutes does not effect complete inversion of the average blackstrap. But since slightly more sucrose was found after heating to 70° for 8–12 minutes than by inversion at 28° it was still doubtful whether 24 hours at this temperature is sufficient for complete inversion.

The second series of experiments was made with 12 samples to settle this question, and also to ascertain the trend of the inversion curve upon longer heating at 60°. The results of this series are given in Table 2.

TABLE 2.—*Effect of temperature and time on acid inversion (Series 2)*  
*Invert Readings*

NO.	60°							28°	
	10 MIN.	12 MIN.	14 MIN.	15 MIN.	16 MIN.	20 MIN.	25 MIN.	24 HR.	29 HR.
15	-20.20	-20.40	-20.40		-20.60			-20.60	-20.60
16	-18.80	-16.40	-16.40		-16.40			-16.40	-16.40
17	-20.60	-20.80	-20.80		-21.00			-21.00	-21.00
18	-18.60			-19.20		-19.40		-19.20	-19.20
19	-17.20			-17.40		-17.40		-17.00	-17.20
20	-18.20			-18.60		-18.40		-18.20	-18.20
21	-17.00			-17.20		-17.40		-17.20	-17.20
22	-16.60			-16.80		-16.60		-16.60	-16.60
23	-18.60					-18.80	-18.80	-18.80	-18.60
24	-19.40					-19.60	-19.60	-19.60	-19.80
25	-19.40					-19.80	-19.80	-19.80	-19.60
26	-17.60					-17.80	-17.60	-18.00	-18.00

*Average Invert Readings*

NO.	60°					28°	
	10 MIN.	14-15 MIN.	15-16 MIN.	20 MIN.	25 MIN.	24 HR.	29 HR.
15-26	-18.27					-18.53	-18.53
15-22	-18.03	-18.35	-18.40			-18.28	-18.30
18-26	-18.07			-18.36		-18.27	-18.27
23-26	-18.75			-19.00	-18.95	-19.05	-19.00

*Average Sucrose (per cent)*

NO.	60°					28°	
	10 MIN.	14-15 MIN.	15-16 MIN.	20 MIN.	25 MIN.	24 HR.	29 HR.
15-26							
J. & G.	36.31					36.50	36.50
J. & McD.	36.30					36.47	36.47
15-22							
J. & G.	37.12	37.36	37.39			37.30	37.32
J. & McD.	37.11	37.35	37.39			37.27	37.28
18-26							
J. & G.	35.82			36.04		35.97	35.97
J. & McD.	35.81			36.03		35.94	35.94
23-26							
J. & G.	34.99				35.14	35.21	35.18
J. & McD.	34.98				35.13	35.18	35.14

The average invert readings obtained after inversion at 28° show that inversion is complete in 24 hours, no significant change being observed upon prolonging the time another 5 hours. The levorotation, as well as the sucrose result, is the maximum attainable by inversion at 28°. At 60° inversion is not complete in 10 or even 12 minutes, and this confirms the result obtained in the first series. When the time is extended to 14-16 minutes, however, the sucrose result rises about 0.1 about that at 28°. If the heating is prolonged still further, to 20 minutes, the excess over the

value at 28° is still around 0.1, but if the heating period is extended to 25 minutes slight destruction of invert sugar is indicated. The top of the inversion curve is rather flat, about the same result being obtained whether the heating at 60° lasts for 14 or for 20 minutes. This is in line with the observation of Jackson and Gillis with pure sucrose, that heating for any period between 8 and 11 minutes does not change the result perceptibly. With impure products the entire process, that of inversion as

TABLE 3.—*Effect of temperature and time on acid inversion (Series 3)*

NO.	INVERT READINGS			
	28° 24 HR.	35° 24 HR.	60° 10 MIN.	60° 20 MIN.
27	-18.60	-18.20	-17.40	-18.00
28	-16.40	-16.00	-16.40	-16.40
29	-17.20	-17.20	-17.00	-17.20
30	-18.80	-18.60	-18.20	-18.40
31	-19.20	-19.00	-18.80	-19.20
32	-19.60	-19.80	-19.40	-19.80
33	-15.80	-15.60	-14.80	-15.40
34	-18.80	-18.80	-18.40	-18.80
35	-17.80	-17.60	-17.00	-17.80
36	-19.40	-19.20	-19.20	-19.60
Av.	-18.16	-18.00	-17.66	-18.06
Average Sucrose (per cent)				
J. & G.	36.92	36.80	36.54	36.84
J. & Mc.D.	36.88	36.77	36.53	36.84

well as that of destruction of invert sugar, proceeds more slowly than with pure sucrose, at one and the same temperature. Zerban<sup>8</sup> had previously shown that the asparagine and aspartic acid occurring in molasses slow up the destruction of levulose by the hydrochloric acid used for inversion.

For further confirmation of the results obtained in the second series of experiments, a third series was run with ten more samples. The inversion was carried out (1) at 28° for 24 hours, which had been found sufficient for complete inversion; (2) at 35° for 24 hours; (3) by the Treasury procedure of heating for 10 minutes at 60°; and (4) at 60° for 20 minutes.

The results (Table 3) again show that heating for 10 minutes at 60° does not give complete inversion, the sucrose being found from 0.3 to 0.4 too low. A temperature of 35° is evidently too high if the inversion is carried

<sup>8</sup> *This Journal*, 14, 172 (1931).

on for 24 hours. Inversion at 60° for 20 minutes gives sucrose results 0.04–0.08 lower than inversion at 28° for 24 hours, whereas in the second series of experiments the result was about 0.1 higher. It would appear from this that in the long run the results obtained by the two procedures will check within 0.1 per cent up or down. In the second series, heating at 60° for 15 minutes also gave a result about 0.1 higher than inversion at room temperature for 24 hours, showing that the average inversion curve is rather flat between 15 and 20 minutes at 60°.

The following conclusions may be drawn from the results reported: (1) The average blackstrap is completely inverted in 24 hours at 28°; (2) heating to 60° for 10 minutes does not give complete inversion, and the time must be extended to 15–20 minutes; (3) heating to 70° for 8 minutes gives a sucrose result checking within less than 0.1 per cent with that obtained after inversion for 24 hours at 28°. It must be understood that these conclusions are valid only for an average Cuban or Puerto Rican blackstrap, and that in individual cases the time given for the different temperatures may either be insufficient for complete inversion or may cause incipient destruction of invert sugar.

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## THE VALUE OF STATISTICS IN THE FORMULATION OF CHEMICAL METHODS\*

By LILA F. KNUDSEN† (Food Division, ‡ Food and Drug Administration, Federal Security Agency, Washington, D. C.)

Most of what follows has been said before; however, very likely the cerebral furrows plowed by the points of these ideas have been leveled by the varying winds of everyday problems and the flood waters of routine work. An attempt is made here to replot some of those furrows with the hope that the contours may be preserved, and erosion checked.

This is not an attempt to give a condensed course in statistics but merely an effort to point out a few of the advantages and uses of the tool. It is left to the reader to point out the disadvantages. Statistics interprets figures, but it may be retorted that "the interpreter is the harder of the two to understand."

Statistics is a tool that can be applied to many fields of endeavor. Up to a decade ago it was used almost exclusively by economists and biometricians. Now, it is being used by psychologists, engineers, manufacturers, the Army and Navy, and a few chemists. In fact, one of the men who has been a prime influence in developing statistical methods was a

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† The author wishes to acknowledge gratefully the encouragement of H. A. Lepper of Food Division, Food and Drug Administration.

‡ W. B. White, Chief

chemist in a Dublin brewery. The reader may have heard of "Student's *t* test" and various papers by "Student." This pseudonym was chosen by William Sealy Gosset because of a clause in his contract with the brewery which prohibited publishing articles under his own name.

In chemistry, statistics furnishes a tool to measure the variability of results, and to point out those results that may be affected by extraneous causes which could be controlled in a more satisfactory manner (such as temperature, type of equipment, manner of interpretation of instructions) and thus help eliminate these causes of variation.

#### QUALITY CONTROL

There are many ways of applying this tool. One of these, namely quality control, has been very successful in munitions making, ordnance, and other manufacturing. Quality control helps locate avoidable faults in manufacturing. This *modus operandi* also governs the Army inspection at Picatinny Arsenal, the Aberdeen proving grounds, and other places, of all manufactured items on which quality can be measured, such as burning time of fuses, weight of an explosive charge, explosive power of detonators, etc.

Dr. Walter A. Shewhart of the Bell Telephone Laboratories and Colonel Leslie E. Simon of the Ordnance Department, U. S. Army, have done more than any other persons to apply statistics to manufacturing processes and ballistics (1, 7, 8). Both have popularized the use of the control chart method.

Possibly the best way to explain the control chart method would be to cite an example of its application. In making even such a small thing as a rivet the manufacturing process has to be tried again and again with minor changes each time, to insure that the width, length, head, etc., of the rivet do not vary more than a certain tolerated amount. This tolerance might be compared with the tolerance or error of a chemical analysis.

In Figure 1, some hypothetical data are used to illustrate the graphic control chart method. Certain types of explosive rivets are more effective if they fit snugly and smoothly into holes that are .001 of an inch larger than their specified shank diameter of .125 of an inch. In the purely hypothetical control chart given in Figure 1, the average, as indicated by the line so labeled, is the average of all previously manufactured rivets, and the control limits or tolerance limits, as indicated by the dotted lines, are obtained from the variation encountered in previous experience (the average plus or minus three times the standard deviation). The plotted points indicate the average measurements of samples of 4 rivets, where one sample of 4 rivets was taken every hour from one machine on different days during production. All of the points here are within the control limits. If a dot falls outside the tolerance lines, the inference is that some extraneous cause or causes have been operating, and the foreman or opera-

tor is told to look for trouble,—and usually finds it. It may be that a different alloy was used for the rivets or a tool in the machine is becoming worn. (There are theoretical reasons behind choosing three standard deviations as a tolerance limit, but what really counts is that *they work*.) Quality control men in munitions, aeronautics, and other manufacturing all over the country and in England have found that if an observation falls outside these limits trouble is brewing or “has brewed.” When all the necessary adjustments on the machinery have been made and the dots are always falling within the tolerance limits the process is said to be “in a state of statistical control,” as in the case of the shank diameter of rivets in Figure 1.

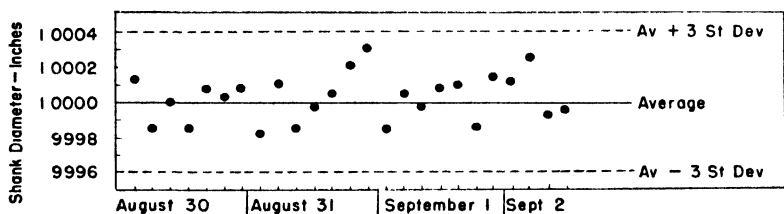


FIG. 1.—CONTROL CHART ON THE AVERAGE SHANK DIAMETER OF EXPLOSIVE RIVETS—HYPOTHETICAL DATA.

Chemical analyses could be “in statistical control” just as much as a manufactured product. In routine analyses of the same product a control chart will point out “trouble”—e.g., when the product is beginning to run below standard or when some difficulty of analysis occurs. For instance, in a series of determinations of moisture content, it was obvious after the plotting of the results that the first of triplicate analyses of a sample was always high no matter what chemist had made the analyses. On looking for the cause, it was found that the grinder (chopping-bowl type) was washed with hot water between samples. Therefore it was cold for the first sample but warm for each subsequent sample, thus causing more evaporation.

Figure 2 illustrates the use of control chart technic on the selenium method as validated by collaborative results given by Klein (4). The results submitted by ten collaborators (A, B, C . . . J) are plotted according to collaborator. When the average and the control limits (the average plus or minus 3 standard deviations) are inserted on the chart, it is obvious that the results of collaborators D and J are essentially different from the results of the other eight collaborators. There are usually very definite causes for such essentially different results: (1) the samples are not strictly identical, (2) the method is not closely enough defined, or (3) the analyst has inadvertently failed to follow the method. In all cases it is desirable to



ferret out and remedy the causes of discrepancies, possibly by sending out new samples from the same lot, cautioning that extra care be taken with the method, and inviting questions and criticism of any nebulous instructions.

This illustration applies the control chart technic to similar samples of one product analyzed by several chemists. A control chart could also be

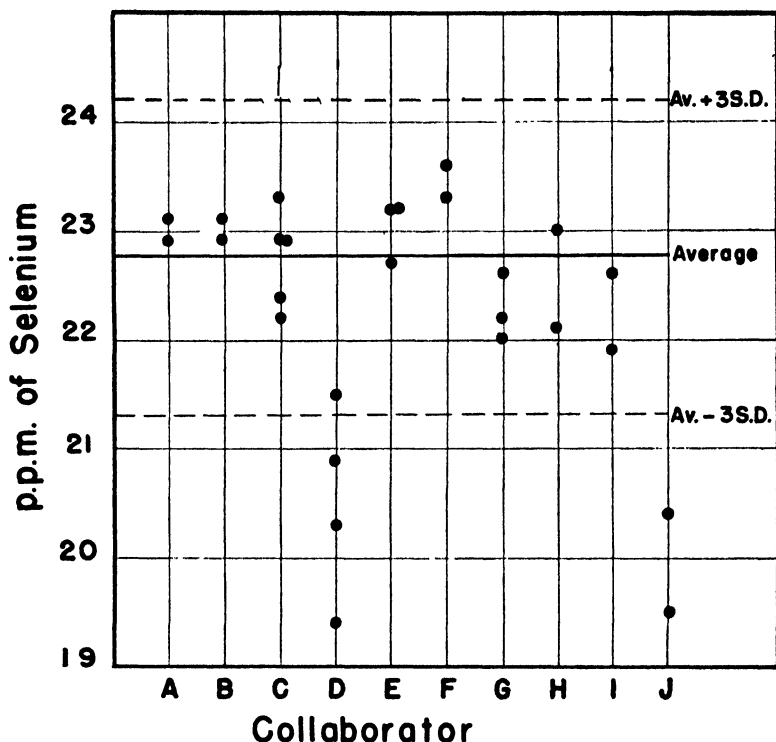


FIG. 2.—CONTROL CHART ON THE RESULTS OF TEN COLLABORATORS ON THE KLEIN SELENIUM METHOD:

kept on the routine analyses of a standardized product over a period of time, or on the differences between duplicates of a single chemist's analyses on several different products. These are merely suggestions on some uses of a control chart; once a control chart method proves its own advantages, the chemist will find many different applications for it.

Since homogeneity forms the basis for the control chart technic, too frequent changes in chemical methods are obviously undesirable. For example, when analyses of a supposedly homogeneous group of samples are being considered, and subsequent samples are to be compared with this group, the earlier and later groups should be analyzed by *identical*

methods. Lepper has pointed out (5) that many methods of analysis are largely empirical, that minor changes merely involve going from one empirical procedure to another, and that minor changes in several successive years may finally produce methods that give results consistently different from those obtained under the original directions. The minor changes to which he referred were those made without a careful trial of their influence on the result. The tool, statistics, will enable an analyst to evaluate the changes in a method, and to gain an idea of the error of the new method and of the tolerances to be allowed. It will also help the analyst to realize the multitudinous sources of error and to so design his experiment or collaborative set-up as to include and evaluate the most important errors. However, in order to use the tool results must be homogeneous as far as possible, that is, the same method must be used. The ideal, of course, is to have enough data on the same method to indicate the need (or lack of it) for a more accurate and/or precise method. To a large extent the policies of the Association of Official Agricultural Chemists are aimed at this target. It has long been recognized that the merit of an analytical method can be measured in terms of the probability of the successful reproduction of its results, and that mere numbers of analyses do not necessarily furnish this information.

#### ACCURACY VS. REPRODUCIBILITY

The tool, statistics, also gives a measure of the accuracy and reproducibility of the results on a method. For the sake of clarity a definition is given of these terms, which are, as all chemists know, two different things. The accuracy of a method is indicative of the agreement between the obtained result and the "true" result, while reproducibility (sometimes called precision) indicates agreement between many obtained results regardless of what the true results may be. Accuracy shows how closely a method measures what it is supposed to measure, while reproducibility shows how closely many measurements agree. Given a sample which is known to contain 100 mg. of iodine, a very accurate and highly reproducible method would give, say, from 99.5 to 100.5 mg. of iodine, while an accurate but poorly reproducible method might give from 95.0 to 105.0 mg. of iodine; an inaccurate but highly reproducible method would possibly give from 85 to 86 mg. of iodine, while a method which is both inaccurate and poorly reproducible might give from 80 to 90 mg. of iodine.

Accuracy can be estimated by comparing the average of several chemical determinations of a given constituent with the amount known to be present. Reproducibility can be given by one figure: the standard deviation (or variation of results around their average). Of course the data used in determining these two estimates—namely, accuracy and reproducibility—should not be obtained from only *one* chemist or only *one* laboratory if they are to be applied to *any* chemist in *any* laboratory.

## TYPES OF ERRORS

To the scientist it is self evident that a single observation would be sufficient for prediction purposes *if* the material and conditions were perfectly controlled and *if* the observations were perfectly made and recorded. Obviously, this is a practical impossibility; no observation can be perfectly repeated. Also, chemists are only human, too, and avoidable errors may creep into their work.

There are several distinct types of analytical errors in analyses of identical material. For the present purpose the over-all error may be divided into three parts. The first type of error involves how closely an analyst can check himself. It is an impossibility for one analyst to check himself perfectly. The second type of error involves how closely one analyst can check another, using the same apparatus in the same laboratory. There may be slight differences in interpretation of instructions or slight differences in technic that cause this type of error. The third type of error involves how closely one analyst can check another at another laboratory. Here again the slight differences in interpretation of instructions enter in, but there are apparently other influencing factors which cause this error to be somewhat larger than the second type.

DESIGN OF EXPERIMENT TO TAKE INTO  
ACCOUNT THESE ERRORS

The collaborative work should be designed to cover all three types of errors. A suggested design is given in Table 1, in which an identical number of replicate analyses are run by each analyst on each method at each laboratory, and individual analyses are reported. In any method it is very desirable for an analyst to be able to check the results of another analyst

TABLE 1.—*Design of collaborative work on identical samples  
comparing two methods*

	ANALYST NO. 1		ANALYST NO. 2	
	METHOD NO. 1	METHOD NO. 2	METHOD NO. 1	METHOD NO. 2
Lab. No. 1	— —	— —	— —	— —
Lab. No. 2	— —	— —	— —	— —
Lab. No. 3	— —	— —	— —	— —
.. .. .	....	....	....	....
.. .. .	....	....	....	....
.. .. .	....	....	....	....

almost as closely as he can check his own results. If this is not the case, frequently there are assignable causes for one type of error being larger than another, and when these causes are pointed out the situation may be remedied, possibly by a slight change in the method itself or a change in the wording of the instructions. This results in minimizing the over-all error and improving the method by increasing its reproducibility.

### SAMPLING AND ITS IMPLICATIONS

The dictionary gives the definition of a sample as "a part of anything presented for inspection, a specimen." According to this definition, the people living in New York City might be considered as a sample of those living in the United States, or a spoonful of milk from the top of an unshaken bottle as a sample of the milk in the bottle. Usually in taking a sample from a batch of material there is a serious attempt at making the sampling procedure random. Sometimes this attempt is so deliberate that the purpose is frustrated. For instance, if one attempts to make a random selection of the numbers of the pages of a book, it would be found that very seldom would the first or the last page be chosen.

One of the chemists in this Administration recalled an account of the choosing of the "Unknown Soldier" for Arlington Cemetery. There were six caskets of unknown soldiers side by side in a tent, and a sergeant was told to select the casket to be sent to Arlington. He entered the tent and after surveying the six caskets placed a red rose on the third as being that of "The Unknown Soldier." The account went on to say that psychological laboratory experiments indicate that almost anyone confronted with this choice would have chosen the third or the fourth casket out of the six and that very seldom, almost never, would a person choose the first or sixth. Therefore this was not a random choice of the Unknown Soldier. It must be remembered that randomness is not synonymous with haphazardness. Randomness means that each unit of whatever is being sampled has an equal chance of occurring in the sample.

Suppose a biochemist feeds two substances to each of two groups of six rats chosen from the same cage. If he gives the first substance to the first six animals he catches, he will not have a random sample of the rats but will have the less lively animals and therefore will be biasing his results at the very start of an experiment. He could have ensured the randomness in some way, possibly by numbering the doses—say 1 to 10—and then putting the doses in random order to be used as the animals were chosen from the cage. The tossing of a die in general indicates a random selection unless the die is loaded or imperfect. The shuffling of a group of numbered disks and the choice of a sample from this group (if the group is relatively small) sometimes gives a fairly good sample. However, the best way of making a truly random choice is to use one of the several published sets of random numbers (3, 9) that offer a series of choices without the use of such auxiliary devices as dice or metal disks.

However, most sampling of agricultural products is designed to be representative rather than random. Nevertheless, a distinction should be made between the two types of samples; the random one where each article of the lot has an equal chance of occurring in the sample and the representative one where each portion of the lot is intentionally represented in the sample.

Sometimes even if the sample is chosen in a truly random manner from a lot of articles, only a small percentage of which is deficient, it estimates the lot as being better than it is. Take certain types of radio tubes, for instance. Given, a carload of radio tubes, 10 per cent of which is known to be defective while 90 per cent is known to be satisfactory, the chances of getting one good radio tube if a *single* tube is taken from the carload are .9. In drawing a sample of two, the chances that both radio tubes will be good are .9<sup>2</sup> or .81. Similarly, the chances of getting ten good tubes in a sample of ten are .9<sup>10</sup> or .35. This means that 35 per cent of the time all ten radio tubes will be satisfactory. In the same manner the chances of getting nine good tubes and one defective tube can be calculated to be .39. In other words, in taking a sample of ten radio tubes from this carload only (100—35—39) or 26 per cent of the time will the sample of ten be poorer than the entire carload while 35 per cent of the time they will be better than the carload.

This bias (overestimating the quality of a lot) occurs only when there is a small percentage of deficiency, and the smaller the percentage of deficiency the greater the bias. Also, as the size of the sample approaches the size of the lot the bias tends to disappear.

The main point to be brought out here is that the existence of this bias should be recognized and the sample considered in that light.

#### A WORD OF CAUTION ON HISTORICAL BACKGROUND OF A METHOD

Recently, in an investigation of methods of cheese sampling a statement was found to the effect that "the best type of sample has been shown to be a wedge of the cheese." Curiosity regarding the results of this sampling experiment led to a search of the records down through such statements as "the best type of sample is a wedge" and finally to the source statement some 30 years previously, "we took a wedge of cheese," with no sampling experiment conducted at all! This type of modification of statements is common in human relations as is perhaps best illustrated by witnesses' testimony in court. It is, therefore, important not to ignore the historical background of the method under consideration.

There are many other more specialized applications of statistics to chemistry that have not been even mentioned here, notably to certain fields of biochemistry. It must be remembered that statistics is just a *common sense* condensation of the multitudinous results of an experiment

into a few figures or graphs. The statistical results naturally can be no better than the experiment itself. Statistics does *not* enable anyone to use the results of one small experiment to draw sweeping generalizations on the entire universe. Further, it *should not* be abused "to conceal damage or inferiority, or to make the article appear better or of greater value than it is."

To conclude, the following is quoted from the "Father of Statistics," Francis Galton:

It is always well to retain a clear geometric view of the facts when we are dealing with statistical problems, which abound with dangerous pitfalls, easily overlooked by the unwary, while they are cantering gaily along upon their arithmetic.

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#### MICROSCOPIC IDENTIFICATION OF CRYSTALLINE SUBSTANCES LISTED IN UNITED STATES PHARMACOPOEIA XII\*†

By GEORGE L. KEENAN (U. S. Food and Drug Administration,  
Washington, D. C.)

Over a considerable period of time in connection with general regulatory work on drugs, the author has collected a large amount of optical crystallographic data on numerous inorganic and organic crystalline substances. In the course of this work it has been apparent that data on such substances are widely distributed in a variety of publications or have never been de-

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† Contribution from Microanalytical Division.

terminated. On numerous occasions the identification of crystalline material through the determination of optical crystallographic properties has been very helpful, especially in instances where the amount of material available was insufficient for the usual chemical tests.

In the descriptions of the compounds included in United States Pharmacopoeia XII, identification is dependent upon certain fixed criteria, such as melting point, boiling point, optical rotation, and chemical test. In many instances optical crystallographic data, especially the refractive indices of these compounds, would be of considerable value from a diagnostic standpoint, and these indices are usually readily obtainable. It is with this thought in mind that the data included in this paper have been compiled as a ready reference for use by analysts interested in the identification of substances capable of treatment by the optical crystallographic technic. It is believed that the use of these data in combination with other usual conventional descriptions of these substances will enable workers in this field to save considerable time and energy in the identification of crystalline substances. Some of the optical data presented in the tables have already appeared in the literature, but a large proportion constitutes unpublished material. In Table 1 the substances are arranged alphabetically with their significant refractive indices, and in Table 2 the substances are arranged according to ascending value of the lowest index of refraction ( $n_\alpha$ ,  $n_e$ , or  $n_\omega$ ). Any significant intermediate refractive index ( $n_i$ ), although not the  $n_\beta$  value, is listed in the  $n_\beta$  column for convenience.

In the selection of optical data, it is obvious that some limit must be set, otherwise the microscopic description could assume the magnitude of a monograph on each substance. The specialist in this field realizes that there is considerable descriptive material common to many crystalline substances that is not necessarily of diagnostic value, and therefore some selection has to be made of those constants that are significant for each substance. In the case of crystalline substances, the refractive indices stand out as the most important constants that can be relied upon in the microscopic identification of the material. To be sure, other optical data may be useful and the worker in this technic may desire to use them. Ultimately, however, the final classification of the substance for purposes of identification will depend upon a determination of the refractive index or indices. Therefore, emphasis has been placed on these values, but where possible the crystal system and general habit or form of the substance have been included.

In view of the fact that the application of the polarizing or chemical microscope to the identification of crystalline substances is becoming more general and several descriptions of the immersion method are already on record in the literature, only a brief description of the general procedure will be given. The details of the technic can be obtained from published articles and textbooks, a list of which is appended.

The microscopic examination of crystalline material may be conveniently divided into three parts, examinations made in plane polarized light (lower nicol in position), in parallel polarized light (crossed nicols), and in convergent polarized light (crossed nicols).

In plane polarized light, the form or habit of the crystalline material will be revealed by direct observation, and the refractive indices can be readily determined. In the immersion method, the crystalline material is immersed in a drop of liquid (usually oily in nature), the refractive index of which has been previously determined with a refractometer. For the determination of the refractive indices, the crystals or crystal fragments of a given substance are successively suspended in liquids of known refractive index, advantage being taken of the fact that the greater the difference between indices of refraction of crystal and liquid, the more prominently the one will stand out from the other. By repeatedly mounting such crystals or fragments in oils of successively lower or higher index, it will be found ultimately that the zone of contact of crystal with liquid becomes practically invisible. At this point, the refractive index of the crystal has been matched with that of the liquid. In the case of substances crystallizing in the isometric (cubic) system, there is only one refractive index, designated as  $n$ . Such substances are not doubly refractive when examined with crossed nicols (parallel polarized light). Substances crystallizing in the orthorhombic, monoclinic, and triclinic systems may show three significant refractive indices, designated as  $n_\alpha$ ,  $n_\beta$ , and  $n_\gamma$ . Substances crystallizing in the tetragonal, hexagonal, and trigonal systems may show two significant indices, designated as  $n_\omega$ , and  $n_e$ . Usually two such values are sufficient for the identification of the substance, but a third value, in the case of biaxial substances, may often be determined.

In parallel polarized light (crossed nicols) it may be ascertained whether the substance is singly or doubly refractive. Singly refracting (isotropic) substances transmit no light when examined with crossed nicols while doubly refracting (or anisotropic) substances do transmit light under the same conditions and exhibit changes from light to dark when the microscope stage is rotated. Extinction angle and sign of elongation may also be determined with crossed nicols.

In convergent polarized light (crossed nicols), it is possible to determine whether the substance is uniaxial or biaxial, this information assisting in determining the orientation and in correctly interpreting the refractive indices obtained.

The specialist in this particular technic will observe that in many instances where the optical-crystallographic data are given for the first time, all of the indices of refraction have not been determined. For instance, in the case of a substance that would be expected to have  $n_\alpha$ ,  $n_\beta$ , and  $n_\gamma$  values, possibly only two will be given, namely  $n_\alpha$  and  $n_\gamma$ . Also, frequently a significant index, but not a conventional one,  $n_i$  (intermediate refractive



TABLE 1.—*Summary of refractive indices and other data by substance*

	CRYSTAL SYSTEM	$n_\alpha$	$n_\beta$ or $n_i$	$n_\gamma$
Acetanilid	Rhombic	1.515	1.620	>1.733
Acetophenetidin*	Monoclinic	1.518	1.574	>1.733
Acetylsalicylic acid	Triclinic	1.505	1.645	1.655
Aminoacetic acid	Monoclinic	1.495	1.615	1.650
Aminopyrine	?	1.520	—	1.732
Ammonium chloride	Isometric	1.643	—	—
Antimony and pot. tartrate	Rhombic	1.620	1.636	1.638
Antipyrine	Monoclinic	1.570	1.694	1.732
Arsenic trioxide	Isometric	1.755	—	—
Ascorbic acid	?	1.483	1.605	>1.695
Atropine	Rhombic	1.550	—	1.595
Barbital†	?	1.445	1.548	1.580
Barbital sodium	?	1.512	—	1.615
Barium sulfate	Rhombic	1.637	1.638	1.649
Benzoic acid	Monoclinic	1.616 (common $n$ )	—	—
Betanaphthol	Monoclinic	1.523	—	1.733
Boric acid	Triclinic	1.340	1.456	1.459
Caffeine‡	?	1.455	1.472 ( $n_i$ )	1.733
Calcium carbonate§	Trigonal			
Calcium lactate	?	1.470	—	1.510
Chloral hydrate	Monoclinic	1.538	1.600	1.602
Citric acid	Rhombic	1.493	1.498	1.509
Cocaine hydrochloride	Rhombic	1.570	1.596	1.618
Codeine	Rhombic	1.543	1.636	1.684
Codeine sulfate	Rhombic	1.561	1.642	1.661
Cupric sulfate	Triclinic	1.514	1.537	1.543
Dextrose	Monoclinic	1.521	—	1.549
Ephedrine hydrochloride	?	1.530	1.603	1.638
Ephedrine sulfate	?	1.540	1.565	1.590
Epinephrine	?	1.555	—	1.733
Ergotamine tartrate	?	1.518	—	1.625
Estradiol benzoate	?	1.586	—	1.632
Estrone	Monoclinic	1.520	1.642	1.692
Eucaïne hydrochloride	?	1.506	1.585	1.645
Eucatropine hydrochloride	?	1.560	—	1.610
Ferrous sulfate	Monoclinic	1.471	1.478	1.485
Homatropine hydrobromide	?	1.603	1.610 ( $n_i$ )	1.645
Lactose	Monoclinic	1.517	1.542	1.550
Magnesium sulfate	Rhombic	1.433	1.455	1.461
Methenamine	?	1.590 (singly refracting)		
Morphine sulfate	Rhombic	1.545	1.620	1.632
Mercury bichloride	Rhombic	1.725	1.859	1.965
Nicotinic acid	?	1.428	1.733 ( $n_i$ )	—
Neostigmine bromide	?	1.560	1.658	1.675
Neostigmine methyl sulfate	?	1.519	1.525	1.580
Nicotinamide	?	1.485	1.734 ( $n_i$ )	>1.733
Pentobarbital sodium	?	1.477	—	1.523
Phenacaine hydrochloride	?	1.518	1.603	1.733

TABLE 1.—Continued

SUBSTANCE	CRYSTAL SYSTEM	$n_\alpha$	$n_\beta$ or $n_s$	$n_\gamma$
Phenobarbital	?	1.557	1.620	1.667
Pilocarpine nitrate	?	1.475	1.588	1.608
Phenolphthalein	Triclinic	1.635	—	1.673
Potassium alum	Isometric	1.450	—	—
Potassium bicarbonate	Monoclinic	1.380	1.482	1.578
Potassium bitartrate	?	1.510	—	1.590
Potassium bromide	Isometric	1.559	—	—
Potassium chloride	Isometric	1.490	—	—
Potassium and sod. tartrate	Rhombic	1.492	1.493	1.496
Potassium iodide	Isometric	1.667	—	—
Potassium nitrate	Rhombic	1.334	1.505	1.506
Procaine hydrochloride	?	1.540	1.556	>1.690
Quinacrine hydrochloride	?	1.522	1.733( $n_s$ )	>1.733
Quinidine sulfate	?	1.565	1.607	1.670
Quinine bisulfate	?	1.555	—	1.620
Quinine hydrochloride	?	1.588	1.615	1.656
Quinine sulfate	?	1.595	1.635	1.690
Saccharin	Monoclinic	1.535	1.690	>1.733
Saccharin sodium	?	1.560	1.642	1.733
Silver nitrate	Rhombic	1.729	—	1.788
Sodium benzoate	?	1.490	—	1.680
Sodium bicarbonate	Monoclinic	1.380	1.500	1.586
Sodium borate	Monoclinic	1.447	1.470	1.472
Sodium bromide	Isometric	1.641	—	—
Sodium carbonate monohyd.	Rhombic	1.420	1.509	1.525
Sodium chloride	Isometric	1.544	—	—
Sodium citrate	Monoclinic	1.470	1.500	1.510
Sodium iodide	Isometric	1.775	—	—
Sodium salicylate	?	1.421	1.445( $n_s$ )	1.678
Sodium sulfate	Monoclinic	1.394	1.396	1.398
Strychnine sulfate	Monoclinic	1.592	1.597	1.661
Sucrose	Monoclinic	1.540	1.567	1.572
Sulfanilamide	?	1.570	1.677( $n_s$ )	>1.733
Sulfapyridine	?	1.680	1.733	>1.733
Sulfapyridine sodium	?	1.590	—	1.700
Sulfathiazole	?	1.674	1.685	>1.733
				(plates)
Sulfathiazole	?	1.605	1.733	>1.733
				(rods)
Talc, purified	Monoclinic	1.539	1.589	1.589
Tartaric acid	Monoclinic	1.495	1.536	1.605
Tetracaine hydrochloride	?	1.488	1.733( $n_s$ )	>1.733
Theophylline	?	1.447	1.695( $n_s$ )	>1.733
Urea¶	Tetragonal			

\* Dimorphic.

† May exist in different phases.

‡ Effloresces in dry air.

§  $n_\epsilon = 1.486$ ;  $n_\omega = 1.658$ .

|| May exist in three crystal phases.

¶  $n_\epsilon = 1.602$ ,  $n_\omega = 1.484$ .

TABLE 2.—*Substances arranged according to ascending value of lowest index*

LOWEST REFRACTIVE INDEX	INTERMEDIATE REFRACTIVE INDEX	MAXIMUM REFRACTIVE INDEX	SUBSTANCE
1.334	1.505	1.506	Potassium nitrate
1.340	1.456	1.459	Boric acid
1.380	1.482	1.578	Potassium bicarbonate
1.380	1.500	1.586	Sodium bicarbonate
1.394	1.396	1.398	Sodium sulfate
1.420	1.509	1.525	Sodium carbonate
1.421	1.445	1.678	Sodium salicylate
1.428	1.733	—	Nicotinic acid
1.433	1.455	1.461	Magnesium sulfate
1.445	1.548	1.580	Barbital
1.447	1.470	1.472	Sodium borate
1.447	1.695	>1.734	Theophylline
1.450	—	—	Potassium alum
1.455	1.472	1.733	Caffeine
1.459	—	—	Ammonium alum
1.470	1.500	1.510	Sodium citrate
1.470	—	1.510	Calcium lactate
1.471	1.478	1.485	Ferrous sulfate
1.475	1.588	1.608	Pilocarpine nitrate
1.477	—	1.523	Pentobarbital sodium
1.483	1.605	>1.695	Ascorbic acid
1.484	—	1.602	Urea
1.485	1.733	>1.733	Nicotinamide
1.486	—	1.658	Calcium carbonate
1.488	1.733	>1.733	Tetracaine hydrochloride
1.490	—	—	Potassium chloride
1.490	—	1.680	Sodium benzoate
1.492	1.493	1.496	Pot. and sod. tartrate
1.493	1.498	1.509	Citric acid
1.495	1.536	1.605	Tartaric acid
1.495	1.615	1.650	Aminoacetic acid
1.505	1.645	1.655	Acetylsalicylic acid
1.506	1.585	1.645	Eucaïne hydrochloride
1.510	—	1.590	Potassium bitartrate
1.512	—	1.615	Barbital sodium
1.514	1.537	1.543	Cupric sulfate
1.515	1.620	>1.733	Acetanilid
1.517	1.542	1.550	Lactose
1.518	1.603	1.733	Phenacaine hydrochloride
1.518	1.574	>1.733	Acetophenetidin
1.518	—	1.625	Ergotamine tartrate
1.519	1.525	1.580	Neostigmine methylsulfate
1.520	—	1.733	Aminopyrine
1.520	1.642	1.692	Estrone
1.521	—	1.549	Dextrose

TABLE 2.—*Continued*

LOWEST REFRACTIVE INDEX	INTERMEDIATE REFRACTIVE INDEX	MAXIMUM REFRACTIVE INDEX	SUBSTANCE
1.522	1.733	>1.733	Quinacrine hydrochloride
1.523	—	1.733	Betanaphthol
1.530	1.603	1.638	Ephedrine hydrochloride
1.535	1.690	>1.733	Saccharin
1.538	1.600	1.602	Chloral hydrate
1.539	1.589	1.589	Talc, purified
1.540	1.556	>1.690	Procaine hydrochloride
1.540	1.567	1.572	Sucrose
1.540	1.565	1.590	Ephedrine sulfate
1.543	1.636	1.684	Codeine
1.544	—	—	Sodium chloride
1.545	1.620	1.632	Morphine sulfate
1.550	—	1.595	Atropine
1.555	—	1.733	Epinephrine
1.555	—	1.620	Quinine bisulfate
1.557	1.620	1.667	Phenobarbital
1.559	—	—	Potassium bromide
1.560	1.658	1.675	Neostigmine bromide
1.560	1.642	1.733	Saccharin sodium
1.560	—	1.610	Eucatropine hydrochloride
1.561	1.642	1.661	Codeine sulfate
1.565	1.607	1.670	Quindine sulfate
1.570	1.694	1.733	Antipyrine
1.570	1.596	1.618	Cocaine hydrochloride
1.570	1.677	>1.733	Sulfanilamide
1.586	—	1.632	Estradiol benzoate
1.588	1.615	1.656	Quinine hydrochloride
1.590	—	—	Methenamine
1.590	—	1.700	Sulfapyridine sodium
1.592	1.597	1.661	Strychnine sulfate
1.595	1.635	1.690	Quinine sulfate
1.603	1.610	1.645	Homatropine hydrobromide
1.616	—	—	Benzoic acid
1.620	1.636	1.638	Antimony and potassium tartrate,
1.635	—	1.673	Phenolphthalein
1.637	1.638	1.649	Barium sulfate
1.641	—	—	Sodium bromide
1.643	—	—	Ammonium chloride
1.667	—	—	Potassium iodide
1.674	1.685	>1.733	Sulfathiazole
1.680	1.733	>1.733	Sulfapyridine
1.725	1.859	1.965	Mercury bichloride
1.729	—	1.788	Silver nitrate
1.755	—	—	Arsenic trioxide
1.775	—	—	Sodium iodide

index, as between  $n_\alpha$  and  $n_\beta$  or  $n_\beta$  and  $n_\gamma$ ), will be recorded. Workers acquainted with this method are well aware that the orientation of the crystal fragments plays an important role in a determination of the conventional refractive indices. Indices measured without regard to orientation would usually be considered worthless. Unfortunately, many organic crystalline substances, especially when they occur in plate-like or irregular fragments, do not furnish the worker with the interference figures upon which he must rely to interpret properly the index values he has obtained. There are cases where it is practically impossible to obtain the proper orientation for the measurement of specific indices. Many substances, also, will persist in assuming one orientation only. Such a feature is significant and valuable for determinative purposes, although possibly disconcerting from a theoretical standpoint. In this collection of new data, therefore, there will be instances where the  $n_\beta$  was not recorded, because interference figures were not characteristic of the substance, or only partially so, and the suitable orientation for the measurement of this index was not obtainable. For the determination of the minimum and maximum index values, those fragments were selected which showed the strongest double refraction. In other words, the lowest index of refraction obtainable on the substance would be considered as  $n_\alpha$  and the highest as  $n_\gamma$ . Fortunately, substances crystallizing in rods and needles and showing parallel extinction, as will be readily appreciated, do not offer such difficulties. In the last analysis, the worker interested in identifying a substance by this technic has to take the substance as he finds it, and the quickest approach to the solution of his problem, consistent with accuracy, will be a dependence on those data that are readily determined, not on those that are theoretically possible yet at the same time impracticable of measurement and too time-consuming, if they can be determined.

In the descriptive list that follows, some crystalline substances have been omitted. These substances are variable in composition and are affected by light, or they become hygroscopic or effloresce under certain conditions. In some instances, the substances omitted are essentially microcrystalline in character, precluding any accurate microscopic study. Data on any of these substances would be considered unreliable and at best only approximate.

#### SUMMARY

The significant refractive indices of ninety crystalline substances listed in United States Pharmacopoeia XII have been recorded. These indices have been arranged in two tables for ready reference in determinative work. A brief description of the immersion method employed in the determination of many of these indices has also been given.

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IRON IN STOCK FEEDS

## COMPARISON OF TITRIMETRIC AND COLORIMETRIC METHODS

By L. S. MARCELLO and C. V. MARSHALL (Plant Products Division,  
Department of Agriculture, Ottawa, Canada)

Two methods are outlined by the A.O.A.C. (1) for the determination of iron in plants: (a) the colorimetric method, which is a direct comparison of the intensity of the ferric thiocyanate color of the unknown solution with that of a standard iron solution; and (b) the volumetric method, in which the unknown iron solution is titrated with titanium trichloride. The latter method is not satisfactory because it is difficult to keep titanium salts in a stable form, as they decompose readily.

The literature regarding the stability of ferric thiocyanate color is somewhat confusing. Hallinan (2) selected thiocyanate in preference to other reagents because it gave a satisfactory color reaction with iron in acid solution. The official method (1) of iron analysis in plants is also based on the color reaction with  $\text{SCN}^-$ . On the other hand, Stugart (3), Winsor (4), Steinhauser and Ginsberg (5), and Cowling and Benne (6) noted that the reddish color of ferric thiocyanate in acidified solutions fades on standing, and that this reaction tends to give low results when this colorimetric procedure is used for determining iron. Rakestraw et al. (7) state that the serious difficulty with the thiocyanate color reaction is the instability of the colored compound, and Cowling and Benne (6) claim that "the present A.O.A.C. colorimetric method for iron in plants is not easily adaptable for use with a photoelectric colorimeter because of the instability of ferric thiocyanate in acidified aqueous solutions." Bradbury and Edwards (8) suggested a new titrimetric method for the determination of ferric iron by titration with mercurous nitrate solution. Kunin (9, 10) applied this method to soils and silicates and found it to compare quite favorably with the stannous chloride method.

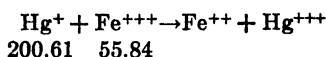
It was decided to apply the Bradbury-Edwards mercurous nitrate method to the determination of the iron content of stock feeds. In this laboratory the colorimetric method (11), whereby the red ferric thiocyanate color is compared to a standard in a Klett colorimeter, has been used. The instrument is costly, and the method involves eye-strain and fatigue.

The details of the titrimetric method used follow:

#### METHOD

##### REAGENTS

(a) *Stock solution*.— $\text{HgNO}_3 \cdot \text{H}_2\text{O}$ , about 0.018 *N*. 1 ml. of 0.018 *N* solution  $\approx$  to 1 mg. of Fe



1 liter of *N* Hg solution  $\approx$  55.84 grams of Fe.

1 ml. of *N* Hg solution  $\approx$  55.84 mg. of Fe.

1 ml. of 0.018 *N* Hg solution  $\approx$   $55.84 \times 0.018 = 1.00$  mg. of Fe.

Weigh out 5.0513 grams of C.P.  $\text{HgNO}_3 \cdot \text{H}_2\text{O}$  into a liter volumetric flask and make to volume in 5%  $\text{HNO}_3$ . Standardize this solution by taking a definite aliquot (50 ml.) and diluting to about 200 ml. Add 15 ml. of *N* HCl. Let precipitate stand overnight and filter on a tared Gooch crucible. Dry at 105°C. to constant weight. From the weight of precipitate calculate the normality of the solution. (This solution has shown no change in titer over a period of three months.)

(b) *Working solution*.— $\text{HgNO}_3 \cdot \text{H}_2\text{O}$ , 0.0018 *N*. Dilute 100 ml. of the stock solution to 1 liter.

1 ml. of 0.0018 *N*  $\text{HgNO}_3 \cdot \text{H}_2\text{O} = 0.100$  mg. Fe.

The strength of this solution has remained constant for eight weeks.

For an alternative method of standardization, titrate the  $\text{HgNO}_3$  against a solution of known iron content.

(c) *Ammonium thiocyanate*.—32 grams of  $\text{NH}_4\text{SCN}$ /100 ml.

##### PROCEDURE

For digestion of the sample use the following procedure as suggested by Marshall et al. (11):

In a 200 volumetric  $\text{P}_2\text{O}_5$  flask, digest 2.0000 grams of sample with 10 ml. of  $\text{HNO}_3$  by boiling gently for 30 minutes, or letting stand overnight. Allow to cool somewhat and add 5 ml. of  $\text{HClO}_4$  (70–72%). Continue digestion by boiling gently until a slight charring occurs when the acid becomes concentrated. Then raise the temperature and complete the digestion until the solution is water white and dense white fumes appear. After cooling, make to volume, allow to settle, and take aliquot.

Place a 20 ml. aliquot representing 0.2 gram of sample in a 200 ml. Berzelius beaker and add 5 ml. of 32%  $\text{NH}_4\text{SCN}$ . Then add the  $\text{HgNO}_3$  solution dropwise from a microburet till the last trace of pink disappears. Shake the solution vigorously after adding each drop of the reagent as the end point is approached. From number of ml. of mercury solution used, calculate percentage of iron in sample.

#### EXPERIMENTAL

The stock feeds analyzed by the colorimetric method and also by the mercurous nitrate method included concentrates of low iron content and mineral supplements containing larger amounts of iron. The results are

TABLE 1.—*Comparison of titrimetric mercurous nitrate method with colorimetric thiocyanate method*

SAMPLE NO.	IRON CONTENT OF STOCK FEEDS	
	COLORIMETRIC METHOD	TITRIMETRIC METHOD
	<i>per cent</i>	<i>per cent</i>
361	0.06	0.06
365	0.93	0.92
366	0.69	0.75
368	0.10	0.11
371	0.04	0.05
372	0.03	0.02
381	0.04	0.05
412	0.24	0.26
440	0.05	0.07
460	0.02	0.03
512	0.07	0.07
515	0.09	0.10
535	0.04	0.04
716	0.07	0.08
718	0.10	0.09
751	0.08	0.09
807	0.05	0.06
811	0.05	0.06
825	0.24	0.24
851	0.04	0.04
857	0.03	0.05
887	0.07	0.09
889	0.04	0.06
891	0.93	0.95
953	0.09	0.10
1102	1.44	1.81
1157	0.05	0.05
1163	0.04	0.04
1218	0.04	0.04
1221	0.09	0.10

recorded in Table 1, which shows that the two methods are in close agreement.

Recovery studies were carried out on nine different samples of stock feeds, which were divided into two groups. To one group a standard iron solution containing 0.12 mg./ml. was added; to the second group a standard iron solution containing 2.47 mg./ml. was added. In all cases 2.000 grams was placed in a digestion flask, the iron solution was added, and the digestion and analyses were carried out as described previously. These results are shown in Table 2. The recoveries shown in the first half of this table vary from 91.7 to 104.2 per cent. It will be seen that the low percentage recovery was obtained where a very small quantity of iron was



TABLE 2.—*Iron recovery studies*

SAMPLE NO.	IRON FOUND in 2 GRAMS	IRON IN SAMPLE	IRON ADDED	IRON RECOVERED	
	mg.	per cent	mg.	mg.	per cent
361	1.28	0.064	0.00	—	—
	1.51		0.24	0.23	95.8
	1.89		0.60	0.61	101.6
	2.51		1.20	1.23	102.5
412	5.16	0.258	0.00	—	—
	5.40		0.24	0.24	100.0
	5.77		0.60	0.61	101.6
	6.34		1.20	1.18	98.3
512	1.46	0.073	0.00	—	—
	1.68		0.24	0.22	91.7
	2.03		0.60	0.57	95.0
	2.70		1.20	1.24	103.3
836	3.32	0.166	0.00	—	—
	3.56		0.24	0.24	100.0
	3.91		0.60	0.59	98.3
	4.51		1.20	1.19	99.2
953	2.00	0.100	0.00	—	—
	2.25		0.24	0.25	104.2
	2.62		0.60	0.62	103.3
	3.21		1.20	1.21	100.8
440	1.38	0.069	—	—	—
	3.83		2.47	2.45	99.1
	6.36		4.94	4.98	100.8
	13.51		12.35	12.13	98.2
512	1.46	0.073	—	—	—
	3.91		2.47	2.45	99.1
	6.50		4.94	5.04	102.0
	13.74		12.35	12.28	99.4
515	1.96	0.098	—	—	—
	4.44		2.47	2.48	100.4
	7.08		4.94	5.12	103.6
	14.39		12.35	12.43	100.6
718	1.86	0.093	—	—	—
	4.42		2.47	2.56	103.6
	6.70		4.94	4.84	98.1
	14.16		12.35	12.30	99.5
825	4.86	0.243	—	—	—
	7.37		2.47	2.51	101.6
	9.81		4.94	4.95	100.2
	17.12		12.35	12.26	99.2

added, and hence experimental error is great. The recoveries shown in the second part of Table 2 range from 98.1 to 103.6 per cent.

### SUMMARY

A method is outlined for the determination of iron in stock feeds. It compares quite favorably with the thiocyanate colorimetric method and has the following advantages over the colorimetric method: (a) It is more time-saving; (b) it is less expensive because no costly apparatus is required; and (c) it is not eye-straining and fatiguing, as is the colorimetric method.

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## MICROSCOPIC DETERMINATION OF WHEAT BRAN IN WHEAT GRAY SHORTS

By EWING E. BROWN and G. S. FRAPS (Texas Agricultural Experiment Station, College Station, Texas)

Microscopic examination has long been accepted as a method for the qualitative detection of adulterants in foods and feeds. It can also be used for quantitative work.

In the quantitative microscopic analyses of feeding stuffs, it is necessary to reduce the particles to sizes that are both small and uniform. A suitable quantity is weighed upon a ruled slide and cleared with the appropriate treatments if necessary, and the characteristic particles are counted. This number is then compared with the count of similar particles from definite quantities of samples of known purity. When suitably modified, the method described here for the determination of wheat bran in wheat gray shorts can also be applied to other ingredients of feeds.

### METHOD

Grind 10 grams of the sample through a 40-mesh sieve in a Wiley Intermediate Mill. In the case of wheat gray shorts, mix thoroughly 1 gram with 9 grams of wheat flour. Weigh .01 gram upon a microscopic slide having a square marked on it about

25×25 mm. Moisten with 3 drops of chloral hydrate solution consisting of equal parts of chloral hydrate, water, and glycerol, and spread evenly over the square. This solution is used for distributing the sample and not for clearing it. By means of a binocular compound microscope with 10×ocular and 1.5 mm. objective and a mechanical stage, count the characteristic particles of wheat bran. Systematically survey the field by means of the mechanical stage. (The wheat bran particles are combinations of structures as shown in the figures on page 1951 of "Winton's Microscopy of Vegetable Foods," Vol. 1 (1932). Count the number of bran particles on three or more slides; then calculate by dividing this number by the average number of particles contained in 0.01 gram of similar mixtures containing 1 per cent of standard bran. The writers found this number to be 2.2.

In order to ascertain the factor to be used for wheat bran, samples of wheat bran considered by the miller to be pure were secured from a number of mills, and mixtures were made containing 0.1 gram of bran and 9.9 grams of flour, and 0.2 gram of bran and 9.8 grams of flour, or 1 per cent and 2 per cent mixtures. The results are given in Table 1. The average count is 21.8 bran particles for 0.01 gram of a 1 per cent mixture, which would be 2.2 for 1 per cent bran at a dilution of 10 per cent, which is used with the wheat gray shorts.

TABLE 1.—*Wheat bran particles in .01 gram of pure wheat bran diluted with wheat flour*

LABORATORY NUMBER	1% MIXTURE			AVERAGE PER 1%	2% MIXTURE			AVERAGE PER 1%
	SLIDE 1	SLIDE 2	SLIDE 3		SLIDE 1	SLIDE 2	SLIDE 3	
67315 Morrison Milling Co. Denton	22	22	23	22.3	45	43	44	22.0
67316 Fant Milling Co. Sherman	21	23	21	21.7	47	41	42	21.7
67317 Burrus Mill and Elevator Co. Fort Worth	20	21	21	20.7	43	45	41	21.5
67318 Seguin Milling Co. Seguin	21	23	22	22.0	44	43	45	22.0
67293 Bewley Mills Fort Worth	23	21	22	22.0	45	42	47	22.3
67294 Liberty Mills San Antonio	31	18	20	23.0	46	44	40	21.7
67295 Pioneer Flour Mills San Antonio	24	20	21	21.7	43	46	40	21.5
67296 H. Dittlinger Roller Mills Co. New Braunfels	22	20	23	21.0	42	45	44	21.8
67345 General Mills* Minneapolis, Minn.	19	23	23	21.7	43	46	43	22.0
67346 Kimball-Diamond Milling Co. Greenville	22	23	21	22.0	45	47	41	22.2
Average				21.8				21.9

\* All other mills located in state of Texas.

TABLE 2.—*Total number of corn bran particles and number that could be mistaken for corn bran in 0.01 gram portion (averages of 3 slides)*

	TOTAL	SIMILAR TO WHEAT BRAN
Special 1	25	3
Special 2	26	3
Special 3	22	2
59496	24	3
60219	25	4
60659	24	2
63683	26	3
Average	25	3

The average count of .01 gram of 1 per cent wheat bran is 22 particles; while the counts of individual slides vary appreciably, ranging from 31 to 18; the averages of 3 slides each of the ten samples range only from 20.7 to 23.0. This shows also that the 10 samples of pure wheat bran were remarkably uniform in bran content. The error would ordinarily be about 5 per cent of the wheat bran present.

In some of the preliminary work, corn meal was used to dilute the wheat gray shorts instead of wheat flour. Corn meal contains particles that may

TABLE 3.—*Bran content by microscopic analysis of commercial feeds called wheat gray shorts*

REGISTRATION NUMBER OF TEST SAMPLE	NAME OF SAMPLE	CRUDE FIBER	BRAN
		<i>per cent</i>	<i>per cent</i>
90743	Wheat gray shorts	4.08	19.0
90764	Wheat gray shorts	6.22	24.0
86831	Wheat gray shorts	4.13	27.5
86792	Wheat gray shorts	4.54	31.0
90664	Wheat gray shorts	6.02	31.5
87123	Wheat gray shorts and screenings	5.78	31.5
90409	Wheat gray shorts and screenings	7.47	35.7
86741	Wheat gray shorts	6.73	38.0
90674	Wheat gray shorts and screenings	6.64	38.2
86571	Wheat gray shorts	8.25	40.0
86559	Wheat gray shorts	8.07	40.5
87114	Wheat gray shorts and screenings	6.23	43.0
86960	Wheat gray shorts	5.25	44.0
87203	Wheat gray shorts and screenings	5.74	45.5
86576	Wheat gray shorts	7.45	49.5
86579	Wheat gray shorts	7.90	55.5
83491	Wheat gray shorts and screenings	6.67	59.0

be mistaken for those of wheat bran unless very thoroughly examined. In order to ascertain how many such particles might be present, several samples of commercial corn meal intended for human food were selected and ground, and 0.1 gram portions were examined. The results are shown in Table 2. With an average of 25 particles to .01 gram of corn meal, 3 particles might be mistaken for wheat bran particles. Therefore corn meal is not a suitable diluent.

### DISCUSSION

If a mechanical stage is not available, a slide with ruled lines would be helpful. Wheat flour was found to spread more easily than corn starch. It is important for the analyst to have standard samples for comparison, in order that he may be familiar with the structure of the particles to be counted.

The content of bran in 17 samples of commercial feeds termed wheat gray shorts and wheat gray shorts and screenings, as determined by the microscopic method, is shown in Table 3, three slides being used for each sample. Although there are some exceptions, the bran content is related to the crude fiber content. The official definition\* adopted by the American Feed Control Officials before 1928 states that a feed should not be called wheat gray shorts if it contains more than 6 per cent crude fiber. Most of the samples (Table 3) that contain more than 33 per cent bran particles also contain more than 6 per cent of crude fiber. It is possible to reduce the crude fiber of commercial wheat products by mixing in wheat white shorts or ground wheat.

### SUMMARY

In order to determine bran in wheat gray shorts by microscopic methods, the sample is ground to a uniform size and diluted with wheat flour, and the bran particles are counted in 0.01 gram weighed on a microscopic slide. The count is converted to percentage of bran by use of a factor obtained in a similar way from pure wheat brans.

The bran so determined is related to the crude fiber content of the wheat gray shorts.

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### ESTIMATION OF CAFFEINE IN COFFEE EXTRACTS

By G. K. CROWELL (Division of Chemistry and Sanitation, New Hampshire State Department of Health, Concord, New Hampshire)

Within the past year a number of brands of soluble coffee extracts have been submitted to this Department for caffeine assay. One brand was labeled as containing equal amounts of coffee extract and added carbohydrates (dextrins, maltose, and dextrose).

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\* Official publication of the Association of American Feed Control Officials (1943).

It was observed that the caffeine recovery from the brands analyzed by means of the Power-Chesnut method,<sup>1</sup> though giving excellent checks on duplicate runs, seemed extremely low, which would indicate either that the labeling was incorrect or that the eight-hour continuous alcohol extraction failed to remove all the caffeine. It was subsequently found that the latter impression was correct.

After the extraction had progressed for a short time it was also observed that the original fine, powdery material became a gummy, impenetrable mass. After two hours of continuous extraction, a portion of the freshly refluxed alcohol was removed from the overflow of the extractors. This sample failed to give a qualitative test for caffeine, which indicates that all the caffeine that could be extracted by this procedure was removed within the first few hours.

It was of interest to determine which of the A.O.A.C. caffeine methods, if any, would give accurate results with products of this character, also to determine why the Power-Chesnut method failed to give a complete extraction.

The methods employed for the estimation of caffeine and the percentage recoveries are given in the table.

Since the actual caffeine contents of the coffee preparations discussed here are not known, the column titled "Percentage Recovery" contains only comparative results that appear to be congruent. This column was included only to aid in the evaluation of each assay.

The term "re-extraction" used in the tables indicates that the sample had originally been extracted for caffeine by the Power-Chesnut method. The residue from the extraction thimble was then removed and air-dried, cooled in a refrigerator, and ground fine in a mortar. Aid in grinding the gummy residue was accomplished by the use of sand. The material was then transferred back to the original extraction thimble and re-extracted for eight hours with fresh alcohol. All the alcohol extracts were combined, and the subsequent procedure followed the Power-Chesnut method. In some cases the grinding and re-extraction were repeated a number of times.

The results (Table 1) show that the Power-Chesnut method does not recover all the caffeine in dried coffee extracts whether added dextrin and dextrose are present or not. With a straight commercial product one grinding and with a commercial product containing added carbohydrates two grindings of the gummy residue with sand followed by re-extraction were required to obtain results comparable to those obtained by the Fendler-Stüber<sup>1</sup> and Bailey-Andrew<sup>2</sup> (tea) methods. With drip-grind coffee added carbohydrates inhibit the extraction and recovery of caffeine, since a re-extraction was required to recover the total amount present.

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<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1940, 195.

<sup>2</sup> *Ibid.*, 198.

TABLE 1

SAMPLE NO.	DESCRIPTION OF SAMPLE	METHOD	PERCENTAGE CAFFEINE		PERCENT-AGE RECOVERY
			GRAV.	NX3.464	
Commercial extract with added carbohydrates					
NPC	A commercial coffee extract purporting to consist of equal parts of coffee extract and added carbohydrates	Power-Chesnut	0.65	0.60	60
NPC-1	Same as NPC plus one re-extraction	Power-Chesnut	0.97	0.91	91
NPC-2	Same as NPC plus two re-extractions	Power-Chesnut	1.07	1.00	100
NPC-3	Same as NPC plus three re-extractions	Power-Chesnut	1.08	1.00	100
NPC-4	Same as NPC extracted for 16 hours	Power-Chesnut	0.66	0.60	60
NFS	Same as NPC	Fendler-Stüber	1.04	0.98	98
NBA	Same as NPC	Bailey-Andrew	1.06	1.01	100
Straight coffee extract					
GPC	A commercial brand of straight coffee extract	Power-Chesnut	1.29	1.21	25
GPC-1	Same as GPC plus one re-extraction	Power-Chesnut	4.78	4.70	99
GFS	Same as GPC	Fendler-Stüber	4.80	4.76	100
GBA	Same as GPC	Bailey-Andrew	4.78	4.74	100
GPC-2	Mixture (50% GPC + 10% dextrin + 40% dextrose)	Power-Chesnut	0.64	0.57	24
GFS-1	Mixture (50% GPC + 10% dextrin + 40% dextrose)	Fendler-Stüber	2.43	2.37	100
GBA-1	Mixture (50% GPC + 10% dextrin + 40% dextrose)	Bailey-Andrew	2.44	2.38	100
GPC-3	Mixture (50% GPC + 25% dextrin + 25% dextrose)	Power-Chesnut	1.66	1.60	67
GFS-3	Mixture (50% GPC + 25% dextrin + 25% dextrose)	Fendler-Stüber	2.44	2.39	100
GBA-2	Mixture (50% GPC + 25% dextrin + 25% dextrose)	Bailey-Andrew	2.44	2.38	100
GPC-4	Same as GPC-3 plus one re-extraction*	Power-Chesnut	1.92	1.87	79
GPC-5	Mixture (75% GPC + 25% dextrose)	Power-Chesnut	0.91	0.85	24
GPC-6	Mixture (50% GPC + 50% dextrose)	Power-Chesnut	0.62	0.58	24
GPC-S	Same as GPC	†(see text)	4.81	4.78	100
Straight coffee					
MPC	Straight coffee ("drip grind")	Power-Chesnut	1.28	1.20	100
MPC-1	Same as MPC plus one re-extraction	Power-Chesnut	1.29	1.20	100
MPC-2	Mixture (50% MPC + 25% dextrin + 25% dextrose)‡	Power-Chesnut	0.43	0.39	65
MPC-3	Same as MPC-2 plus one re-extraction	Power-Chesnut	0.62	0.59	98
MPC-4	Same as MPC-2 plus two re-extractions	Power-Chesnut	0.62	0.59	98
MFS	Same as MPC	Fendler-Stüber	1.26	1.18	98
MFS-1	Same as MPC-2	Fendler-Stüber	0.63	0.58	97
MBA	Same as MPC	Bailey-Andrew	1.28	1.22	100
MBA-1	Same as MPC-2	Bailey-Andrew	0.63	0.60	100

\* Very gummy residue, impossible to grind thoroughly.

† Combined Fendler-Stüber and Power-Chesnut methods.

‡ The residue from an 8 hour extraction was slightly gummy.

The agreement of the maximum recovery on re-extractions by the Fendler-Stüber and Bailey-Andrew methods and the agreement of results by these two methods under the various conditions tried indicate that they give results that represent the total caffeine present. Sample GPC-S was subjected to a combination of the Fendler-Stüber and Power-Chesnut methods for the estimation of caffeine. The sample was treated as outlined in the Fendler-Stüber method to the point where the chloroform extract was evaporated on the steam bath, and the final traces of chloroform were removed with a current of air. The dry residue was dissolved in warm alcohol and then subjected to the Power-Chesnut method, where the alcohol extract is added to the heavy magnesium oxide in suspension with water. This procedure was expected to give good recovery, and to

determine whether a purer caffeine extract could be obtained. This aim was accomplished as excellent agreement was obtained between the gravimetric and nitrogen assay methods.

#### SUMMARY

(1) The Power-Chesnut method failed to extract all caffeine present in the coffee extracts examined.

(2) The results indicate that when the tentative Fendler-Stüber method and the official (tea) Bailey-Andrew method are applied to coffee extracts they give complete recovery of caffeine.

### FACTORS AFFECTING DETERMINATION OF ACID- AND BASE-FORMING QUALITY OF FERTILIZERS\*†

By H. R. ALLEN and LELAH GAULT (Kentucky Agricultural Experiment Station, Lexington, Ky.)

This paper is a report of an investigation of certain factors that affect the method of determining the acid- and base-forming quality of fertilizers.

In the 1942 A.O.A.C. collaborative work on the proposed method for determining basicity of material coarser than 20 mesh<sup>1</sup>, very good check results were obtained on 2 of the 3 samples sent to collaborators. On Sample 1a, however, to which had been added coarser-than-20-mesh dolomite equivalent to 150 pounds of calcium carbonate per ton, the average result of the collaborators was only 127 pounds. The washed, coarser-than-20-mesh portion of this sample contained the equivalent of about 750 pounds of calcium carbonate per ton. When a 1-gram portion and 30 ml. of normal hydrochloric acid were used in the determination, there was sufficient excess acid to react with all the carbonate in the sample, but it was found that the reaction did not always proceed to completion because of the low acid concentration. A back titration was obtained with 0.5 *N* sodium hydroxide, which resulted in a low basicity figure. The presence of undecomposed carbonate was shown by effervescence when the residue, after filtration, was treated with hydrochloric acid.

(1) *Relationship between amount of dolomite present in sample and amount of excess acid required to react with all the carbonate.*—A sample of mixed fertilizer (0.5 gram) was mixed with each of the weights of a dolomite shown in Table 1, and the basicity of the mixtures was determined by the A.O.A.C. method,<sup>2</sup> with variations in the amounts of normal hydro-

\* This investigation, made in connection with a project of the Kentucky Agricultural Experiment Station, is published by permission of the Director.

† Presented before the Division of Fertilizer Chemistry at the 106th Meeting of the American Chemical Society, Pittsburgh, Penn., and released for publication in *This Journal*.

<sup>1</sup> *This Journal*, 26, 68 (1943).

<sup>2</sup> *Methods of Analysis*, A.O.A.C., 1940, 37, 59.



chloric acid and in the use of molar and 0.5 molar sodium carbonate. The dolomite was ground to pass through a 0.5 mm. sieve. Digestions were made in beakers placed in a water bath heated by gas. The bottoms of the beakers were above the level of the water. The temperature of the solutions in the beakers during digestion was between 92° and 94°C. The basicity due to the mixed fertilizer in the mixture was determined by analysis as 50 pounds of calcium carbonate equivalent per ton, and the basicity

TABLE 1.—*Basicity results (pounds  $\text{CaCO}_3$ /ton) when  $M$  and  $0.5 M \text{Na}_2\text{CO}_3$  and  $N \text{HCl}$  were used in indicated quantities (digestion in water bath)*

0.5 GRAM OF MIX PLUS DOLOMITE	$M \text{Na}_2\text{CO}_3$		$0.5 M \text{Na}_2\text{CO}_3$		
	30 mL. $N \text{HCl}$	35 mL. $N \text{HCl}$	20 mL. $N \text{HCl}$	25 mL. $N \text{HCl}$	20 mL. $N \text{HCl}^*$
<i>gram</i>					
0.15	352	367	340	350	360
0.20	447	470	442	455	460
0.25	547	560	547	555	570
0.30	642†	660	625†	645	680
0.35	692†	762	720†	755	775
0.40	800†	855	815†	845	885

\* 0.25 gram of mixed fertilizer plus .075, .10, .125, .15, .175, and .20 gram.

† Effervescence in residue when  $\text{HCl}$  was added.

TABLE 2.—*Basicity results (pounds  $\text{CaCO}_3$ /ton) when mixture was digested on hot plate*

0.5 GRAM OF MIX PLUS DOLOMITE	INDICATOR TITRATION		GLASS ELECTRODE TITRATION	
	$M \text{Na}_2\text{CO}_3$	$0.5 M \text{Na}_2\text{CO}_3$	$M \text{Na}_2\text{CO}_3$	$0.5 M \text{Na}_2\text{CO}_3$
<i>gram</i>				
0.30	660	657	660	662
0.35	760	755	766	761
0.40	860*	850†	865*	869†

\* 31 ml.  $N \text{HCl}$  used.

† 21 ml.  $N \text{HCl}$  used.

of the dolomite as approximately 2050 pounds per ton. Calculation of the basicity of the mixtures was made on the basis of a 1-gram sample. Theoretical basicity of any determination may be calculated from the basicity of the mixed fertilizer plus added dolomite as follows: 50 pounds plus weight of dolomite in grams times 2050. One group of determinations was made on samples, each of which contained 0.25 gram of the mixed fertilizer and one-half the weights of dolomite indicated in Column 1 of Table 1.

Similar basicity determinations were made with digestion on a gas-heated hot plate, the mixtures indicated in Table 2 being used. The temperatures of the solutions were 98° to 99°C., and 1 ml. of additional

normal hydrochloric acid was added in the determination where 0.4 gram of dolomite was used.

The effect of reducing the volume of water added was investigated by reducing the total volume of the digestion solution from 80 to 50 ml., as indicated in Table 3. Digestions were made on the sand bath; in the water bath with a current of carbon dioxide-free air passing through the solution for 30 minutes; and in the water bath, the solution being stirred three times during the digestion.

Lower basicity results may be expected on samples containing more than 500 pounds of calcium carbonate equivalent per ton when a 1-gram portion and 30 ml. of normal hydrochloric acid are used. Mixed fertilizers

TABLE 3.—*Effect of volume of water used on basicity results (pounds  $\text{CaCO}_3$ /ton)*

0.5 GRAM OF MIX PLUS DOLOMITE	M $\text{Na}_2\text{CO}_3$ 30 ML. N HCl AND 20 ML. $\text{H}_2\text{O}^*$	0.5 M $\text{Na}_2\text{CO}_3$	
		20 ML. N HCl AND 30 ML. $\text{H}_2\text{O}^\dagger$	20 ML. N HCl AND 30 ML. $\text{H}_2\text{O}^\dagger$
gram			
0.30	653	653	648
0.35	758	753	745
0.40	848	848	838

\* Digestion on sand bath

† Digestion in water bath. Current of  $\text{CO}_2$ -free air passed through solution for 30 minutes during digestion.

‡ Digestion in water bath. Solution stirred 3 times during digestion.

usually do not contain this much dolomite, but the washed coarser-than-20-mesh portion may contain as much or more if the fertilizer contained 100 pounds or more of coarser-than-20-mesh dolomite per ton. Somewhat higher results were obtained when a 0.5 gram sample was used, and this seems preferable for the higher amounts of dolomite. When determinations of basicity run more than 500 pounds per ton, the residue should be tested for undecomposed carbonate. Higher results were obtained when the digestion was made on the hot plate. This appeared to be due to the  $5^\circ$  to  $7^\circ$  increase in temperature of the solution. Some later determinations, made on a steam bath in which the temperature of the solutions was as high as on the hot plate, indicate that digestion on the steam bath is as effective as on the hot plate. Reducing the volume of water and passing a current of carbon dioxide-free air through the solution during digestion did not give maximum results, especially with the 0.4 gram sample of dolomite.

(2) *Comparison of ashing temperatures of  $500^\circ$  and  $600^\circ\text{C}$ .*—The A.O.A.C. method<sup>2</sup> specifies ashing the sample at  $500^\circ$ – $600^\circ\text{C}$ . after evaporation to dryness with 10 ml. of molar sodium carbonate solution containing 50 grams of sucrose per liter. The ashing should volatilize all the

nitrogen in the sample. The extent of the volatilization at these temperatures was investigated by ashing duplicate samples of the mixtures indicated in Table 4 at 500° and 600°C. and determining the nitrogen content of the ashed samples. Molar and 0.5 molar sodium carbonate were used (the sucrose content of the 0.5 molar sodium carbonate solution was 25 grams per liter). Ashing was made in an electric muffle equipped with a Wheelco Capacitrol temperature regulator.

TABLE 4.—*Per cent nitrogen in ash after ignition at 500° and 600°C.*

SAMPLE 0.5 GRAM OF MIX PLUS GRAMS DOLOMITE	500°C.		600°C.	
	M Na <sub>2</sub> CO <sub>3</sub>	0.5 M Na <sub>2</sub> CO <sub>3</sub>	M Na <sub>2</sub> CO <sub>3</sub>	0.5 M Na <sub>2</sub> CO <sub>3</sub>
<i>gram</i>				
0.15	0.51	0.11	0.11	0.06
0.25	0.51	0.11	0.18	0.06
0.40	0.53	0.14	0.15	0.06
0.3 gram (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> and 0.7 gram Superphos	0.02	0.01	0.01	0.03
0.38 gram NaNO <sub>3</sub> and 0.62 gram Superphos	0.12	0.21	0.04	0.04
0.19 gram tankage and 0.81 gram Superphos	0.31	0.19	0.01	0.01

A temperature of 500°C. was not high enough to volatilize all the nitrogen in the sample, but a temperature of 600°C. was satisfactory for this purpose. A 0.5 molar sodium carbonate with 25 grams per liter of sucrose was more effective at 500°C. and at 600°C. than the molar sodium carbonate with 50 grams of sucrose per liter. Nitrogen from ammonium sulfate was almost completely volatilized at 500°C., but appreciable amounts of nitrogen from sodium nitrate and tankage were found in the samples ashed at 500°C.

(3) *Comparison of 0.25 and 0.5 gram sample in determination of basicity of dolomite and of C. P. calcium carbonate.*—Basicity determinations on the dolomite used in this work and on a C. P. sample of calcium carbonate were made; 0.25 and 0.5 gram samples, each with molar and 0.5 molar sodium carbonate, were used. The basicity of the dolomite was checked by determining the calcium and magnesium contents by the A.O.A.C. methods<sup>3</sup> and calculating each to the calcium carbonate equivalent. By this procedure a basicity of 2060 pounds of calcium carbonate equivalent per ton was obtained. Results are reported in Table 5.

The 0.5 gram sample seems preferable for determination of basicity of

<sup>3</sup> *Methods of Analysis*, A.O.A.C., 1940, 34, 47; 36, 53.

dolomite or calcium carbonate. Slightly higher than theoretical results for the calcium carbonate were obtained when the 0.25 gram sample was used. For a 0.5 gram sample, 35 ml. of normal hydrochloric acid with molar sodium carbonate, or 25 ml. of normal hydrochloric acid with 0.5 molar sodium carbonate, should be used. The determination of basicity of the dolomite when a 0.5 gram sample was used checked closely with the calcium carbonate equivalent of the dolomite from analysis of the calcium and magnesium content.

(4) *Recovery of added coarser-than-20-mesh dolomite in mixed fertilizers as affected by sampling.*—A major factor in the determination of basicity

TABLE 5.—*Comparison of 0.25 and 0.5 gram samples in determination of basicity of dolomite and C. P. calcium carbonate (results in pounds CaCO<sub>3</sub>/ton)*

	<i>M</i> Na <sub>2</sub> CO <sub>3</sub>		0.5 <i>M</i> Na <sub>2</sub> CO <sub>3</sub>	
	0.5 GRAM*	0.25 GRAM	0.5 GRAM†	0.25 GRAM
Dolomite‡§	2050	2100	2050	2080
Calcium carbonate	2005	2020	1995	2020

\* 35 ml. *N* HCl used.

† 25 ml. *N* HCl used.

‡ 2060 pounds by determination of calcium and magnesium content.

§ Theoretical CaCO<sub>3</sub> equivalent of dolomite is 2171 pounds per ton.

of dolomite coarser than 20-mesh in mixed fertilizers is the difficulty of obtaining uniform portions from the unground sample. Unground samples of mixed fertilizer containing varying amounts of dolomite, the particle size of which was between 10 and 20-mesh, were prepared. Each sample of 500 grams was thoroughly mixed, and four 100 gram portions were weighed out. The sample was placed on a piece of oilcloth and each 100-gram portion was selected from 12 to 15 places as follows: A piece of light sheet aluminum 3 by 3 inches was turned up on 2 opposite sides to form a flat boat with open ends. The boat was slid under the sample, one end was closed by holding a flat piece of aluminum firmly against it, and the contents were transferred to the weighing container. After each 100-gram portion had been weighed the sample was re-mixed and spread out, and the operation was repeated for second, third, and fourth portions. The 100-gram portions were wet-sieved through a 20-mesh sieve, and the basicity of the coarser-than-20-mesh portion was determined as directed in the 1942 A.O.A.C. report.<sup>1</sup> From similar samples of 400 grams each, 4 portions were selected by means of the Jones sample riffle, and the basicity of the coarser-than-20-mesh portion was determined.

Results were duplicated within 10 pounds for coarser-than-20-mesh material up to 100 pounds, and within 15 pounds for material of 150 pounds per ton, as shown in Tables 6 and 7.

TABLE 6.—*Recovery of added coarser-than-20-mesh dolomite in mixed fertilizers (pounds CaCO<sub>3</sub>/ton). Hand sampled*

SAMPLE NUMBER	WRIGHT OF >20-MESH PORTION IN GRAMS	ADDED >20-MESH DOLOMITE	BASICITY OF ASH	
			WHOLE >20-MESH PORTION	DUE TO MIXED FERTILIZER
1a	29.8	25	43	13
b	26.7	25	37	13
c	26.7	25	36	13
d	22.0	25	36	13
2a	17.6	50	55	5
b	18.1	50	50	5
c	16.4	50	52	5
d	15.0	50	47	5
3a	29.4	100	103	12
b	29.2	100	113	12
c	26.3	100	105	12
d	20.9	100	96	12
4a	20.3	150	167	7
b	19.1	150	164	7
c	18.5	150	157	7
d	19.4	150	152	7

TABLE 7.—*Recovery of added coarser-than-20-mesh dolomite in mixed fertilizers (pounds CaCO<sub>3</sub>/ton). Sampled by Jones riffle*

SAMPLE NUMBER	WRIGHT OF >20-MESH PORTION IN GRAMS*	ADDED >20-MESH DOLOMITE	BASICITY OF ASH	
			WHOLE >20-MESH PORTION	DUE TO MIXED FERTILIZER
5a	24.1	50	62	13
b	30.0	50	64	13
c	24.1	50	60	13
d	26.2	50	55	13
6a	28.1	100	108	13
b	32.5	100	122	13
c	25.7	100	110	13
d	24.6	100	107	13
7a	18.2	150	155	7
b	21.6	150	167	7
c	19.1	150	164	7
d	17.8	150	149	7

\* Weight of washed coarser than-20-mesh portion divided by weight of whole sample.

## SUMMARY

The factors affecting the determination of acid- and base-forming quality of fertilizers investigated were the following:

- (1) Relationship between the amount of dolomite and the amount of excess acid required to neutralize all the dolomite.
- (2) Comparison of the use of molar and 0.5 molar sodium carbonate in the determination.
- (3) Comparison of ashing temperatures of 500°C. and 600°C.
- (4) The recovery of added coarser-than-20-mesh dolomite in mixed fertilizers as affected by sampling.

## MICROSCOPIC IDENTIFICATION OF SODIUM AND POTASSIUM BY MEANS OF THEIR CRYSTALLINE PICROLONATES

By WILLIAM V. EISENBERG and GEORGE L. KEENAN\* (Food and Drug Administration, Federal Security Agency, Washington, D. C.)

In testing various reagents for their suitability to a new and highly specialized use, it was observed that picrolonic acid readily yielded a crystalline precipitate with several compounds produced by various manufacturers. This was subsequently learned to be due to the presence of a sodium salt used in the preparation of these products. Although potassium compounds yielded a crystalline precipitate of similar habit, it was found that the crystalline picrolonates of sodium and potassium could be differentiated by their optical crystallographic properties. The reaction seemed to offer a quick method for the identification of sodium and potassium ions.

The reagent used consisted of a 0.5 per cent solution of picrolonic acid in 50 per cent ethyl alcohol. A drop of this reagent was applied directly to the material or to a drop of the test solution on a microscopic slide and gently warmed. Immediately there formed small masses of yellow rods and needles, frequently occurring in circular aggregates of needles readily visible at 100 $\times$  (Figure 1). In the case of solid material the needles appear to emerge directly from the small fragment of test material. With test solutions the needles may form at the edge of the drop, and it is necessary to stir while warming for the purpose of inducing crystallization throughout the drop.

The material formed on the slide is allowed to dry at room temperature. After drying, the yellow crystalline precipitate is transferred to another slide for examination with the polarizing microscope and the immersion method.<sup>1</sup> In the case of sodium, the small rods and needles that had separated from the circular aggregates were distinctly doubly refracting,

\* Contribution from Microanalytical Division.

<sup>1</sup> Keenan, George L., *This Journal*, 15, 626 (1932).

with parallel extinction and negative elongation when examined with crossed nicols (parallel polarized light). The commonly occurring refractive index ( $n_\alpha$ ) was found to be 1.616, and it was shown when the rods and needles were oriented with their long dimension parallel to the vibration plane of the lower nicol (lengthwise). The maximum refractive index ( $n_\gamma$ ) is greater than that of methylene iodide (1.734) and is shown crosswise on the rods and needles. Twenty sodium salts furnished this test without any difficulty.

Potassium forms a crystalline compound of the same habit with picrolonic acid, and if reliance were placed wholly on the objective appearance of the sodium and potassium salts when examined microscopically, one could be readily confused for the other. Optically, however, the salts are not identical and can be differentiated readily by the immersion method and examination with the polarizing microscope. The potassium salt prepared similarly for examination exhibited inclined extinction, instead of parallel, as in the case of the sodium salt. The elongation is negative for both salts. The minimum refractive index ( $n_\alpha$ ) for potassium picrolonate is much lower than that for the sodium salt, being 1.505. An intermediate index ( $n_i$ ), 1.519, also is shown frequently. The maximum refractive index, like that for the sodium salt, is higher than that for methylene iodide (1.734). Twenty-two potassium salts furnished this test as easily as did the sodium compounds.

*Significant Optical Data for Sodium and Potassium Picrolonates*

<i>Substance</i>	<i>Habit</i>	$n_\alpha$	$n_i$	$n_\gamma$	<i>Extinction</i>	<i>Elongation</i>
Na picrolonate	Yellow needles, rods	1.616	—	>1.734	Parallel	Negative
K picrolonate	Yellow needles, rods	1.505	1.519	>1.734	Inclined	Negative

The test was found sufficiently sensitive to yield a copious crystalline precipitate with one drop of 0.2 per cent sodium nitrate (i.e., of the order of 28 micrograms of sodium), and likewise with one drop of 0.2 per cent potassium nitrate (i.e., of the order of 38 micrograms of potassium).

$\text{Ca}^{++}$ ,  $\text{Ba}^{++}$ ,  $\text{Sr}^{++}$ ,  $\text{NH}_4^+$ ,  $\text{Li}^+$ ,  $\text{Pb}^{++}$ ,  $\text{Cu}^{++}$ , and  $\text{Mg}^{++}$  yield precipitates with picrolonic acid and should be removed when testing for sodium and potassium, since, as is generally the case in microchemical tests, the presence of interfering ions will affect the form of the crystalline precipitate. Calcium and lithium form very fine wavy trichites; barium, a yellow amorphous precipitate; lead, a yellow flocculent mass of minute needles; and magnesium compact circular masses of minute needles, all unsuitable for optical study. Ammonium and strontium form crystalline picrolonates showing different habit and optical properties than those of sodium and potassium. Copper yields a somewhat similar crystalline precipitate as sodium and potassium but can be differentiated by its optical properties.

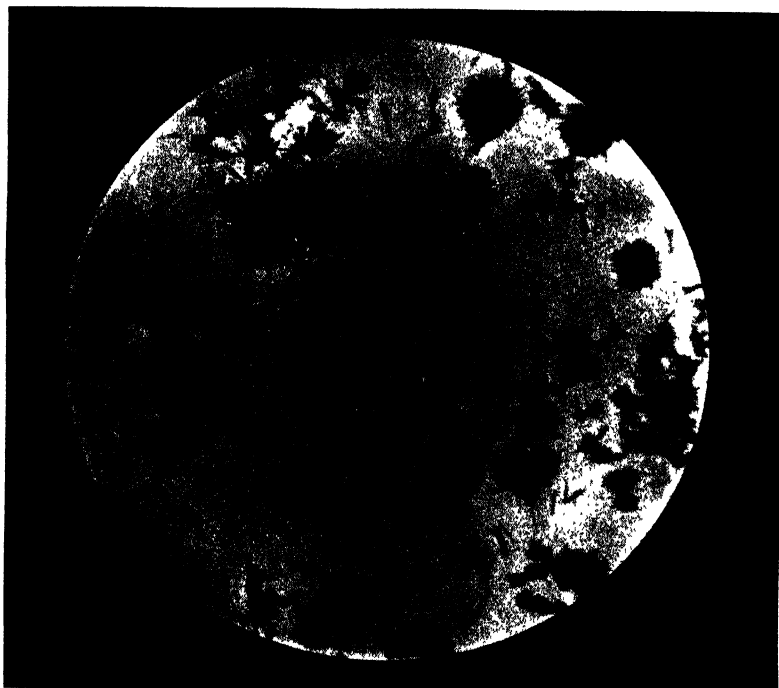


FIG. 1. TYPICAL HABIT OF CRYSTALLINE PICROLONATES  
OF Na AND K ( $\times 75$ )





The procedure described is most effective on sodium and potassium salts present in dry physical mixtures encountered in the examination of food and drug products where the unknown material may be isolated as minute fragments under a low-power, wide, field binocular microscope. This offers a quick and direct means for the positive identification of sodium and potassium ions.

In many chemical microscopic methods total reliance is placed upon the formation of a characteristic crystalline precipitate. When this precipitate is characteristic enough so that little or no interference may result from the presence of other substances this method is of great value. However, instances will arise, as in the present study, where the habit alone is not significant as a differentiating characteristic. In such cases a determination of the optical constants, if possible, serves as a specific means of identification.

#### SUMMARY

A chemical microscopic test for the identification of sodium and potassium has been described, and optical crystallographic constants have been given for differentiating the sodium and potassium salts formed with picrolonic acid.

#### MOISTURE IN POTATO STARCH\*

By W. L. PORTER and C. O. WILLITS (Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture, Philadelphia 18, Pa.)

Although numerous studies on moisture in cereals and feeds have been conducted, they have not provided a fully satisfactory method for making this determination, and the results are widely divergent. The relatively few data available on moisture in potato starch are especially inconclusive.

An investigation was made of most of the better known methods for the estimation of moisture in agricultural products. The procedures were evaluated, and certain of the methods were modified in order to adapt them to the determination of the moisture content of potato starch. These studies included distillation procedures, the Karl Fischer chemical method, and such oven-drying methods as gravity convection, mechanical convection, and vacuum.

Drying by heat is the procedure commonly used for the determination of moisture in starch. The official method of the German starch trade (14) specifies that five grams of starch be dried in a shallow dish for 1 hour at 50°C. and then for 3 hours at 120°C. The first drying period is intended to

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\* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 27, 28, 1943.

prevent gelatinization of the starch; unless the moisture content is high, however, gelatinization will not take place. Schmorl (10) pointed out that in grains (malt) dried above 105°C. there is a noticeable decomposition of organic material, and it is his opinion that this organic material is essentially starch.

Nelson and Hulett (7) postulate that decomposition of cereals heated in a vacuum occurs at widely different rates at all temperatures, but that until a definite temperature is exceeded the loss from decomposition is slight. They assume that at this temperature such loss becomes measurable and increasingly greater. When plotted, the point where this loss begins is indicated by a break in the moisture-temperature curve. This point does not necessarily show the total amount of moisture present in the substance, but it does show the temperature to which the substance can be safely heated. Then, by extrapolating the first portion of the curve to the critical temperature of water, the total moisture content may be estimated.

Sprockhoff (14) claimed an accuracy of 0.2–0.4 per cent for a method in which 10 grams of starch is supported in a pan suspended by a wire passing through the top of an oven and attached to one arm of a balance beam. The oven is kept at 90°–100°C. for 15–20 minutes and then at 140°–150°C. for 25–35 minutes. The Brabender moisture tester used in some of the drying studies reported in this paper is a modification of this device.

Keller and Keller (4) state that it is possible to dry grain and flour at 160°C. for 10 minutes or at 150°C. for 20 minutes in a Trinkler oven, or at 160°C. for 21 minutes or at 150°C. for 25 minutes if an air oven is used. Eva, Milton, and Geddes (2) show that the 130°C. air-oven, the gravity convection Brabender oven, and the DeKhotinsky oven give values for moisture that are similar to the vacuum oven (about 0.1 per cent lower).

The oven methods employed by Sair and Fetzer (8, 9) in their investigation of moisture determination in corn yielded results that ranged from a low value for the air oven at 110°C. to a high value for the vacuum oven at 100°C. They demonstrated that corn dried in the air oven still contained moisture by the fact that use of  $P_2O_5$  in conjunction with the air oven gave a value comparable to that obtained with the vacuum oven.

The official method of the English starch trade specifies the use of the Carter-Simon moisture tester, which follows the principle of higher temperature for short drying periods. The oven is equipped with a chimney to give a more rapid circulation of air in the drying compartment, which is relatively small in proportion to the size of the drying dishes and to the chimney displacement. A 5 gram sample is dried for 15–30 minutes at 155°C.

Snyder and Sullivan (11, 12, 13) reported that when flour is dried dextrinization takes place, followed by a marked change in the solubility of the carbohydrates, in the color of the flour, and in other physical and

chemical characteristics. The dextrinization of the starch, together with dehydration of the proteins during drying, and elimination of water in both cases, account for the anomalous moisture results commonly observed in the determination of moisture in flour.

Distillation procedures for moisture determination in which toluene, xylene, light petroleum, and other solvents lighter than and immiscible with water are used have been found satisfactory for some materials. Sair and Fetzer (8, 9) found that distillation with toluene and with benzene yielded results that were similar and that compared favorably with their established moisture content. Alexander (1) reported the use of carbon tetrachloride, a liquid heavier than water, with a special trap so designed that the column of carbon tetrachloride would support the condensed water column. This has certain advantages over the lighter liquids in that it is nonflammable and keeps the sample from lying on the bottom of the flask, thus preventing superheating and charring.

It may require hours to distil over all the water with the lower-boiling liquids, while the use of high-boiling liquids leads to decomposition of the material with the production of water. Tausz and Rumm (15) and Fairbrother and Wood (3) describe methods of distillation with tetrachloroethane, another solvent that is heavier than water and has a higher boiling point than xylene or toluene, but they do not consider the possible decomposition of the carbohydrate material. Moreover, they use as their standard of comparison gravity convection-oven drying at 98.5°C. This is an empirical procedure, as it measures the water liberated at that temperature, but does not necessarily measure the total moisture content of the sample.

Tucker and Burke (16) report that distillation of most cereal products with tetrachloroethylene gives values that are the same in a few cases but usually are higher than those obtained by drying at 98.5°C. Lampe (5), who investigated starchy materials, reports that his results show the tetrachloroethylene procedure to be suitable for routine moisture determinations.

In the investigation of the use of the Karl Fischer reagent for the chemical determination of moisture in regenerated cellulose, paper, and wood, Mitchell (6) reports that the method gives a more nearly complete recovery of moisture than do the oven-drying methods. He obtained consistently higher results with the Karl Fischer reagent than by drying the same material at 102°C. to constant weight. It does not necessarily follow, however, that these results represent the actual moisture content, since the oven-drying procedure at 102°C. may not have removed all the moisture.

#### METHODS USED

Three samples of potato starch and one of rice starch were obtained. Each sample was thoroughly mixed by rolling it on a sampling cloth and

then stored in moisture-tight Mason jars. The following discussion is mainly concerned with the results obtained with Sample 1, potato starch.

*Mechanical Convection-Oven Methods.*—Two types of mechanical convection ovens were used, a Brabender moisture tester and a Precision floor model.

The Brabender moisture tester, an air oven with forced circulation, has a balance incorporated in the apparatus in such a manner that the sample can be weighed without removing it from the drying chamber. As the loss in weight is read directly in per cent on an illuminated scale, it can be measured at any interval of time for a given temperature, thus eliminating errors inherent in methods that require removal of the sample from the oven, cooling before weighing, and reheating to constant weight. Thus the rate of loss in weight caused by heat at any given temperature can be followed closely. Duplicate 5 gram ( $\pm 0.005$ ) samples were weighed into tared, shallow aluminum pans (85 mm.  $\times$  15 mm.) and placed in the drying chamber, which had been regulated to a given temperature. The loss in weight was noted at definite time intervals until a constant reading was obtained. This procedure was repeated for each temperature under consideration. All samples used in this study were weighed initially at the same time and stored in a desiccator containing no desiccant.

In addition to the Brabender, a Precision floor-model mechanical convection oven and tared aluminum moisture dishes (50 mm.  $\times$  25 mm.) with tight-fitting covers were used. Tests were made, either in duplicate or in triplicate, on 1–5 gram samples. The dishes were uncovered during the drying period. All the samples for each drying temperature were weighed and placed in the oven simultaneously, and each sample was removed after a definite time interval, cooled, and weighed. Since by this procedure the time required to reach constant weight at the temperature under consideration was determined without repeated periods of heating and cooling, the only opportunity for error was in the single cooling process.

*Gravity Convection-Oven Methods.*—A Precision table-model gravity convection oven was used for many of the air-oven tests. The same moisture dishes and weights of sample were used as were employed in the mechanical convection oven, and the method of obtaining the degree of drying was the same as with the Precision mechanical convection oven.

The Carter-Simon tests were made on 5 gram ( $\pm 0.005$ ) samples, which were weighed out in the shallow, open, Carter-Simon moisture pans at the same time. Each sample was run through the oven at a different rate. Thus the time that each sample was in the drying chamber was varied. This procedure was repeated for each temperature of the experiment.

*Vacuum-Oven Drying Method.*—A Weber vacuum oven was used. The pressure in the drying chamber was maintained at less than 5 mm. of mercury by use of a Welch "Duo-seal" pump. The same dishes, weight of

sample, and method of controlling errors were used as for the precision mechanical convection-oven drying method.

*Distillation Methods.*—The apparatus used for solvents heavier than water was the Hercules moisture tester (1); for solvents lighter than water, a modified Bidwell-Sterling apparatus was used. Twenty-gram samples were weighed into the distillation flasks, and sufficient solvent (100 ml.) was added to fill the receiver and cover the sample. The receivers had a capacity of 5 ml. graduated in 0.05 ml. Distillation was continued until the volume of the trapped water remained constant (3–5 hours).

*Chemical Method.*—The Karl Fischer chemical method for the determination of moisture was used. The reagent was prepared according to the Mitchell (6) modification of the original Karl Fischer formula. One-gram samples were extracted with 10 ml. of water-free methanol for 8 hours in 125 ml. Erlenmeyer flasks covered with a piece of rubber dental dam. The titration was carried out by inserting the buret tip through a pinhole in the rubber, thus preventing moisture in the laboratory air from coming in contact with either the reagent or the reaction mixture, thus making possible a sharp end point and reproducible results.

#### DISCUSSION

The percentage loss in weight of potato starch recorded by the Brabender moisture tester becomes greater with successively higher temperatures (Table 1). The rate of loss at each temperature is high in the beginning and falls off rapidly until the weight of the sample remains constant. In Figure 1 the percentage loss in weight is plotted against time. The curve for 120°C. lies just above that for 100°C., and the curve for each temperature is higher than the one for the temperature immediately below it. These curves show that the total amount of volatile material liberated is dependent upon the temperature used. At the higher temperatures a light-brown discoloration appears, indicating an alteration in some of the samples during the time necessary to establish the constancy of weight. This alteration, however, does not affect the weight of the sample, as was demonstrated by a test run at a temperature above that for any run illustrated in Figure 1. Table 2 reports the data on potato starch dried in the mechanical convection oven for varying lengths of time at 180°C. The spectral reflectance of each of the samples was measured with a General Electric recording spectrophotometer, freshly deposited magnesium oxide being used as the standard. Parallel iodine tests were made on water extracts of the samples to indicate changes in composition and solubility. In Figure 2 values of  $-\log R$ , where  $R$  equals reflectance at 436 millimicrons, are plotted against  $\log T$  (time in minutes). Increasing values of  $-\log R$  correspond with increasing intensity of color as viewed by the eye. From Figure 2 and Table 2 it may be observed that no noticeable change

TABLE 1.—*Loss in weight of potato starch<sup>a</sup> dried in Brabender moisture tester at various temperatures for different periods of time*

100°C.		120°C.		135°C.		150°C. <sup>b</sup>		155°C. <sup>b</sup>		180°C.		170°C.	
TIME	LOSS IN WT.	TIME	LOSS IN WT.	TIME	LOSS IN WT.	TIME	LOSS IN WT.	TIME	LOSS IN WT.	TIME	LOSS IN WT.	TIME	LOSS IN WT.
min.	per cent	min.	per cent	min.	per cent	min.	per cent	min.	per cent	min.	per cent	min.	per cent
2	3.15	2	3.40	2	4.40	1	5.20	1	9.20	1	2.25	1	2.40
5	6.25	4	7.40	4	9.05	2	7.50	2	10.95	2	5.85	2	7.20
9	10.20	6	10.30	6	12.00	3	10.85	3	11.50	3	8.90	3	10.80
12	11.95	8	12.20	7	13.00	4	11.70	4	13.80	4	11.20	4	13.25
15	13.00	10	13.30	8	13.80	5	12.85	5	14.60	5	12.90	5	14.80
18	13.85	12	14.20	9	14.35	6	13.90	6	15.20	6	14.10	6	15.90
21	14.35	14	14.80	10	14.85	7	14.60	7	15.60	7	15.10	7	16.40
24	14.80	16	15.35	11	15.30	8	15.30	8	16.00	8	15.70	8	16.50
27	15.25	18	15.60	12	15.50	9	15.70	9	16.25	9	16.15	9	16.75
30	15.30	20	15.70	13	15.75	10	16.15	10	16.35	10	16.35	10	16.80
33	15.50	21	15.80	14	15.90	11	16.25	11	16.50	11	16.55	11	16.80
36	15.55	22	16.05	15	16.00	12	16.45	12	16.60	12	16.65	12	16.85
39	15.60	24	16.05	16	16.20	13	16.45	13	16.60	13	16.70	13	16.85
42	15.85	26	16.10	17	16.30	14	16.45	14	16.60	14	16.70	14	16.85
45	15.85	28	16.15	18	16.30	15	16.45	15	16.65	15	16.70	15	16.90
48	15.90	30	16.20	19	16.30	16	16.50	16	16.65	17	16.80	16	16.85
51	15.95	32	16.20	20	16.30	17	16.55	17	16.65	19	16.80	17	16.85
54	15.95	34	16.20	21	16.30	18	16.60	18	16.70	22	16.85	20	16.90
57	16.00	36	16.35	22	16.35	19	16.60	19	16.70	25	16.85	25	16.90
60	16.00	38	16.40	23	16.35	21	16.60	20	16.70	30	16.85	30	16.90
63	16.00	40	16.35	25	16.40	24	16.60	22	16.70	42	16.85	40	16.90
66	16.00	42	16.30	27	16.35	26	16.60	25	16.70	52	16.85	50	16.90
74	16.00	45	16.40	29	16.35	29	16.55	30	16.70	60	16.85	60	16.90
95	16.00	50	16.35	31	16.40	30	16.55	40	16.70				
122	16.00	60	16.30	33	16.40	34	16.60	50	16.70				
164	16.00			35	16.40	39	16.60	60	16.70				
				39	16.35	44	16.65	70	16.70				
				44	16.40	50	16.65	80	16.70				
				50	16.35	60	16.55						
				60	16.40								

<sup>a</sup> Sample 1.<sup>b</sup> Sample was stored in empty desiccator for 2 days before the determination was made, but was weighed at the same time as all other samples.

in the reflectance of the starch and no change in solubility occurred until the constant-weight period was reached. During the constant-weight period, a gradual increase in the discoloration ( $-\log R$ ) was noted, paralleled by a gradual increase in the solubility of the starch, as indicated by the iodine reaction. Although discoloration and solubilization were proceeding during the plateau period, they did not affect the weight of the sample, as indicated by the constancy of weight over a period of nearly four hours. After four hours of heating, a more drastic breakdown or alteration occurred, accompanied by a further loss in weight, a sharp rise in the rate of discoloration, and the production of erythrodextrins, as was shown by the production of red color in the iodine test. Thus loss in weight occurred only after much longer time intervals than were necessary to establish the constant weight value. Since this was true when drying at 180°, it must be true likewise for drying at lower temperatures, as illus-

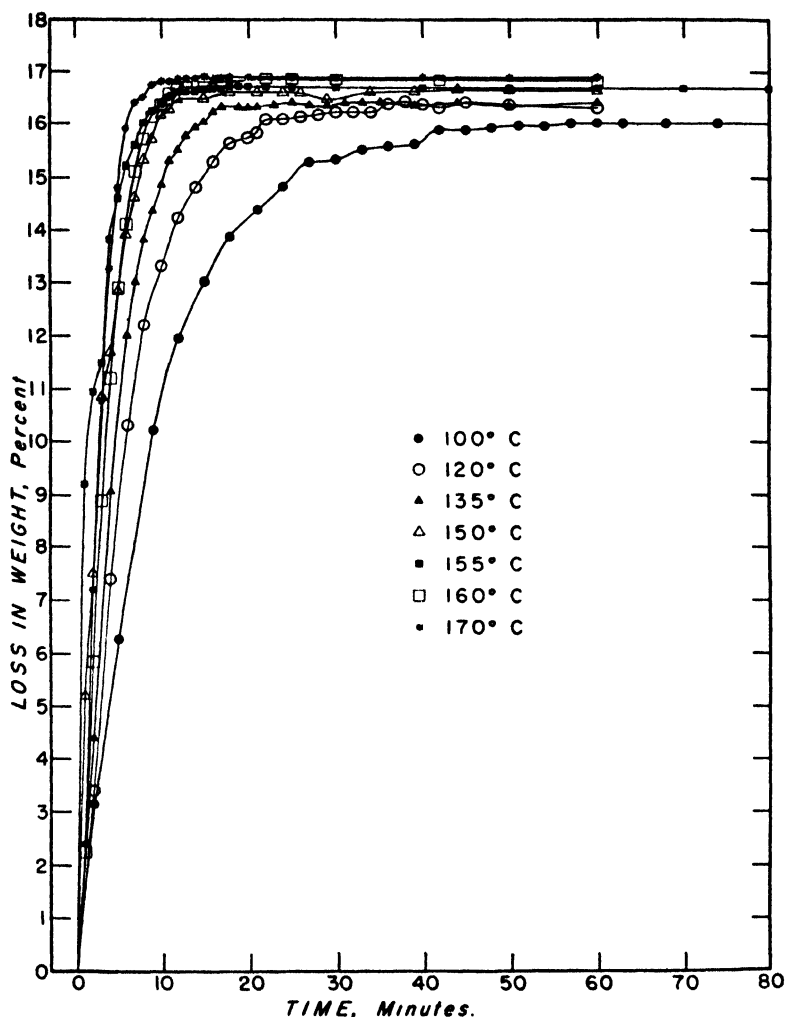


FIG. 1.—LOSS IN WEIGHT OF POTATO STARCH (SAMPLE 1) DRIED IN THE BRABENDER MOISTURE TESTER AT VARIOUS TEMPERATURES FOR DIFFERENT PERIODS OF TIME.

trated graphically in Figure 1. These data also confirm Nelson and Hulett's observations (7) that decomposition occurs at widely different rates and that until a definite time is reached the loss from decomposition is slight, even at a high temperature, but that after this point the loss from decomposition becomes increasingly greater.

If the loss that occurs in reaching constant weight is plotted against the respective temperature for each isotherm of Figure 1, a moisture-temperature curve such as that shown in Figure 3 results. The low temperature end of this curve is rather steep, the loss in weight being sensitive to slight dif-



TABLE 2.—*Effect of drying potato starch (Sample 1) at 180°C. in mechanical convection oven*

TIME	LOSS IN WT.	COLOR	REFLECTANCE AT 436 M $\mu$	IODINE TEST
<i>minutes</i>	<i>per cent</i>		<i>per cent</i>	
Control	—	White	90.3	—Colorless
1	0.78	White	90.2	—Colorless
4	7.08	White	90.2	—Colorless
8	13.57	White	89.9	—Colorless
12	16.17	White	89.4	—Colorless
20	16.67	Very light brown	87.4	+Faint green
30	16.67	Light brown	80.0	+Green
<i>Hours</i>				
1	16.69	Brown	69.5	++Blue
2	16.66	Brown	61.2	++Blue
4	16.71	Brown	51.4	++Purple
24	17.51	Dark brown	9.7	+++Red
48	18.89	Dark brown	5.8	++++Red

ferences in temperature. As the temperature increases, the curve flattens out, the loss in weight apparently becoming less sensitive to temperature variations. Then above a certain temperature, the curve again becomes steep, and smaller changes in temperature produce greater changes in weight. Thus, the curve is divided into two distinct portions, which can

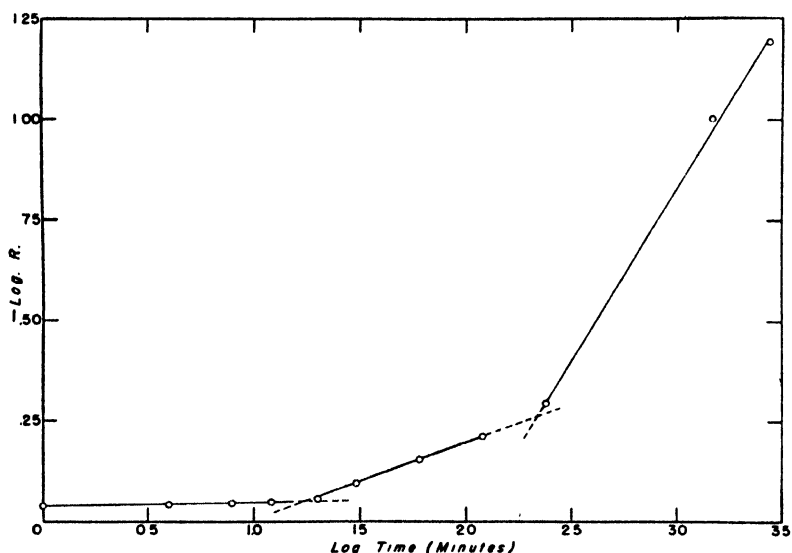


FIG. 2.—REFLECTANCE OF POTATO STARCH (SAMPLE 1) DRIED AT 180°C. IN A MECHANICAL CONVECTION OVEN FOR VARIOUS PERIODS OF TIME.

be extended until they intersect, the intersection being apparently the maximum temperature for the flat portion of the curve.

Any method for the determination of moisture based on the steep portion of the curves of Figure 1 or the upper portion of the curve of Figure 3 is subject to considerable error produced by relatively small changes in time (Figure 1) or in temperature (Figure 3). This may account for the inconclusive data reported in the literature for moisture content of potato starch, since most of the standard methods specify periods of time or temperatures that fall on the steep portions of the respective curves. Therefore, a more nearly reproducible method for the determination of

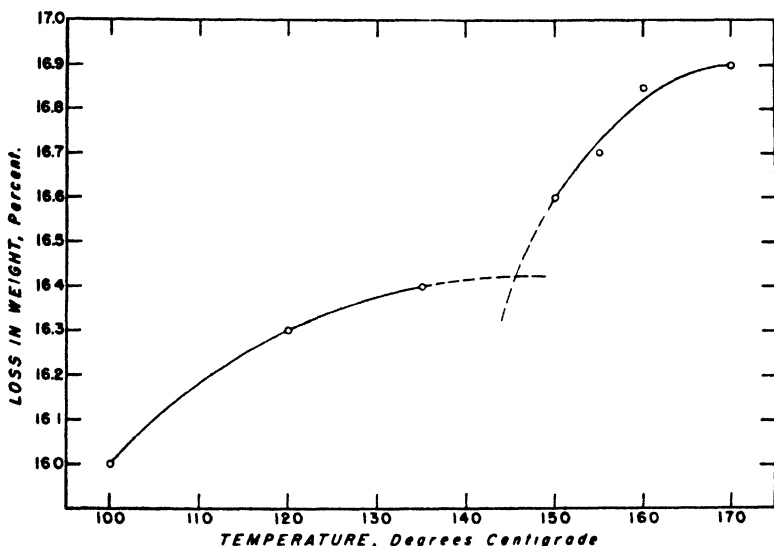


FIG. 3.—LOSS IN WEIGHT OF POTATO STARCH (SAMPLE 1) DRIED AT VARIOUS TEMPERATURES IN BRABENDER MOISTURE TESTER.

moisture in potato starch would consist of the use of a temperature indicated on the flattest portion of the moisture-temperature curve and of a time sufficiently long to insure that constant weight has been reached. With the Brabender moisture tester the method would consist of heating at a temperature of 135°–145°C. for 30–60 minutes or to constant weight. For Sample 1 this would give a moisture content of 16.43 per cent.

Nelson and Hulett (7) suggested that the first portion of a similar curve be extrapolated to the critical temperature of water to obtain the true moisture content of the sample. When their data were plotted, an apparent straight line was obtained, which was interpreted to indicate that the increase in loss of moisture was directly proportional to the increase in drying temperature. A critical study of their data, however, reveals that the slope at each point on the curve is not a constant, which excludes the pos-

sibility of a straight line. This is further proved by replotting their data on a larger scale, which results in a curve very similar to that in Figure 3. The extrapolation of a curve requires many more points and a much higher degree of accuracy than the extrapolation of a straight line. Since these conditions are experimentally unattainable under the present circumstances, it does not seem possible to make this extrapolation. Furthermore, the flattening of the curve before the break suggests that little or no moisture remains in the sample. Hence, there seems to be no need to extrapolate, and the percentage indicated by the break has been taken as the moisture content.

The cause of the greater loss in weight at temperatures higher than the

TABLE 3.—*Loss in weight of starch heated to constant weight at various temperatures in Brabender moisture tester*

TEMPERATURE	LOSS IN WEIGHT (%)			
	POTATO STARCH			RICE STARCH
	SAMPLE 1	SAMPLE 2	SAMPLE 3	
°C.				
90				7.95
100	16.00			8.25
110		10.95	6.25	8.70
120	16.30	11.35	6.35	9.55
130				9.55
135	16.40	11.40	6.45	
140				9.60
145		11.45	6.55	
150	16.60	11.50	6.65	9.75
155	16.70			
160	16.85	11.75	6.90	9.95
170	16.90	11.90	7.10	10.10

break is undetermined, but it may be decomposition or a more deep-seated dehydration of the starch molecule. Since the slope of the curve just above the break is steep and the curve below the break is nearly flat, it may be considered that the extent of decomposition or deep-seated dehydration at temperatures up to the break is negligible.

That the temperature at which the break in the moisture-temperature curve occurs is unaffected by the initial moisture content was confirmed with two other samples of potato starch obtained from different sources and having different moisture contents. The results show that for potato starch the point at which the break occurs can be duplicated at 145°C. ( $\pm 2^\circ$ ). These data are reported in Table 3 and illustrated graphically in Figures 4 and 5. As a check, a new aliquot of each of Samples 2 and 3 was dried to constant weight in the Brabender moisture tester at 145°C. The cross on Figures 4 and 5 represents the percentage loss for each aliquot.

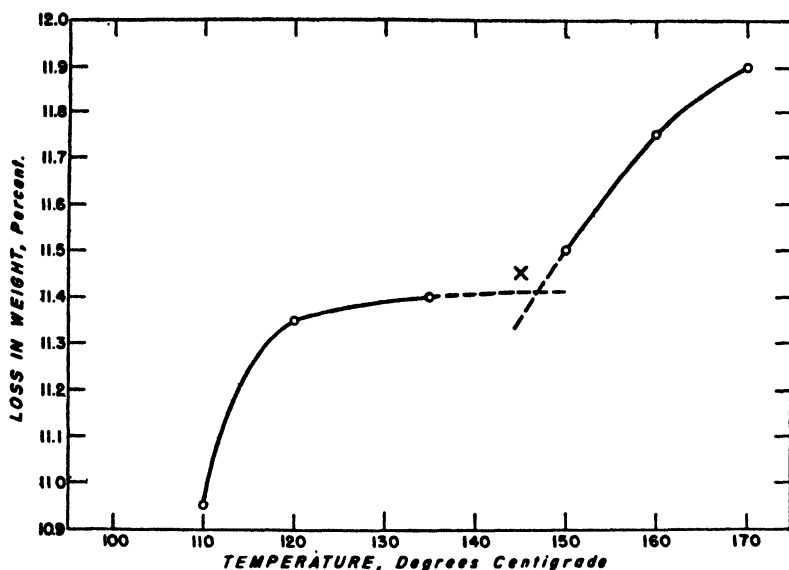


FIG. 4.—LOSS IN WEIGHT OF POTATO STARCH (SAMPLE 2) DRIED AT VARIOUS TEMPERATURES IN BRABENDER MOISTURE TESTER.

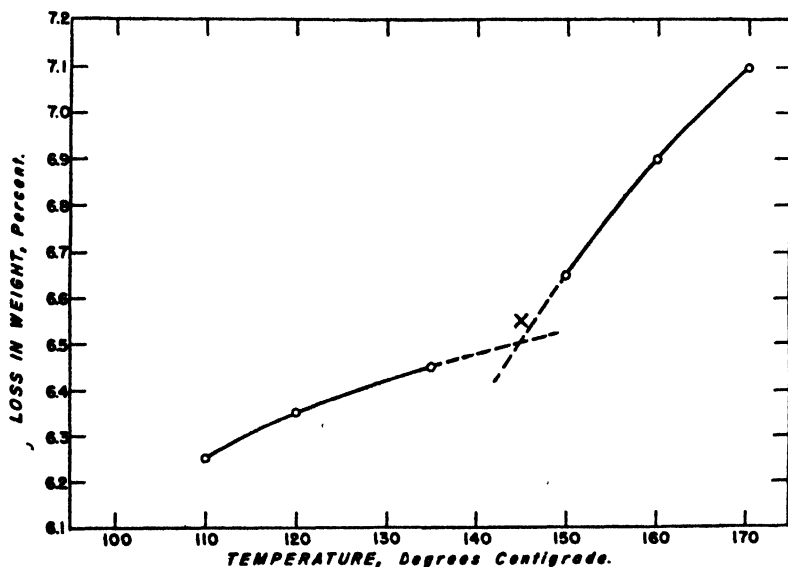


FIG. 5.—LOSS IN WEIGHT OF POTATO STARCH (SAMPLE 3) DRIED AT VARIOUS TEMPERATURES IN BRABENDER MOISTURE TESTER.

These values check the loss in weight at the break within the reading accuracy of the instrument ( $\pm 0.05$  percent). If the type of starch is different, however, a new temperature range will be found. For example, a curve for

rice starch\* results in a flat portion with a range of 120°–135°C. (Figure 6).

Hence, there is proposed a method for the determination of moisture in potato starch that consists in drying the sample to constant weight in the Brabender moisture tester or some similar apparatus at a temperature range of 135°–145°C.

Now that a basic method has been found that gives reproducible results in the determination of moisture in potato starch, it should be possible to employ any other method that will duplicate these moisture values within

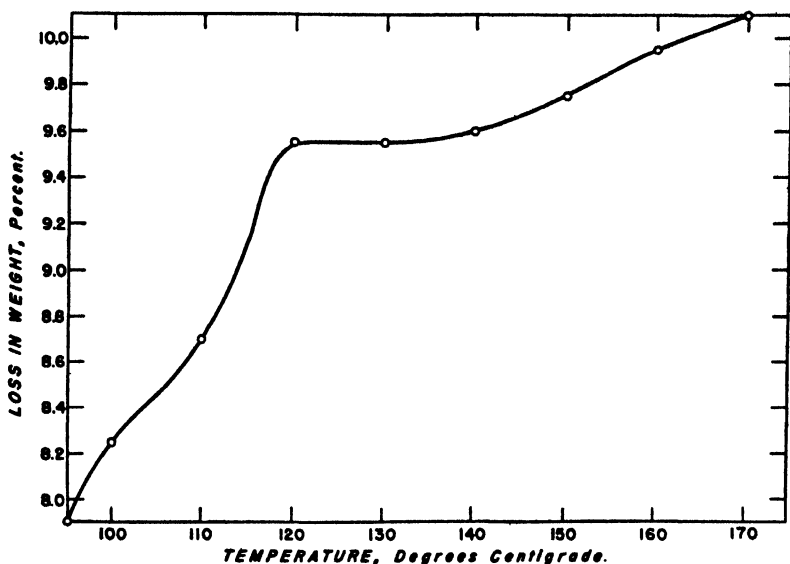


FIG. 6.—LOSS IN WEIGHT OF RICE STARCH DRIED AT VARIOUS TEMPERATURES IN BRABENDER MOISTURE TESTER.

the limit of error of the specific procedure in question. Therefore, the following experiments were planned and carried out to find which of the common methods fulfill these conditions.

When starch was dried in a gravity-convection air oven at 100°C., the loss in weight after 24 hours of drying never quite reached the value obtained in the mechanical convection air oven at 100°C. Furthermore, the rate of drying in the mechanical convection oven was much more rapid than that in the gravity convection oven (Table 4). In neither case, however, did the value reach that established by the basic procedure mentioned above (see Figure 3). Table 4 also shows that vacuum-oven drying of starch under a pressure of approximately 5 mm. of mercury at 80°C. resulted in a moisture value in close agreement with that obtained by the basic method and that constant weights were reached after 22–24 hours.

\* Supplied by G. E. Hilbert of the Northern Regional Research Laboratory, Peoria, Ill.

TABLE 4.—*Loss in weight of potato starch (Sample 1) dried at various temperatures in ovens of different types*

TEMPERATURE	AIR OVEN		MECHANICAL CONVECTION OVEN		VACUUM OVEN (PRESSURE CA. 5 MM.)		CARTER-SIMON OVEN	
	TIME	LOSS IN WT.	TIME	LOSS IN WT.	TIME	LOSS IN WT.	TIME	LOSS IN WT.
°C.	hours	per cent	hours	per cent	hours	per cent	minutes	per cent
80					1.5 22 24	15.48 16.35 16.48		
100	{ 1 2 3 24	13.78 15.20 15.60 15.61	1 2 22 67	15.84 15.98 15.92 16.07	1 5 24	16.47 16.56 16.58		
135	{ 2 5 22	16.39 16.58 16.58	1 12	16.43 16.53			15 30 60	14.66 16.24 16.24
150	{ ½ 1 2 3	16.66 16.66 16.65 16.77	½ ½ 1 3	16.44 16.60 16.71 16.65			10 15 20 30	13.86 16.47 16.52 16.52
180			minutes { 1 4 8 12 20	0.78 7.08 13.57 16.17 16.67				
			hours { ½ 1 2 4 24 48	16.67 16.69 16.66 16.71 17.51 18.89				

Vacuum drying at 100°C. gave a slightly higher value, but the weights were constant after 5 hours. Drying at 100°C. by any of the other methods gave lower values. At 135°C. the air-drying methods used, with the exception of the Carter-Simon, gave results comparable with those of the proposed method.

On heating the starch to constant weight at higher temperatures (150°C. and above), the loss in weight was greater than that caused by loss of moisture. Quick approximations of the moisture content can be made, however, by heating at one of these high temperatures for a length of time that will give a loss in weight comparable to the moisture content estab-

TABLE 5.—*Moisture in potato starch (Sample 1) determined by distillation with various immiscible solvents*

SOLVENT	BOILING POINT	H <sub>2</sub> O
	°C.	per cent
Carbon tetrachloride	76	15.50
Toluene	110–111	16.20
Xylene	137–140	16.83
Tetrachloroethane	146	16.40

lished by the basic method. Heating must be stopped at the expiration of this specific time, since the time selected would fall on the steep portion of a curve similar to those in Figure 1. For example, when samples were heated at 150°C. in the mechanical-convection air oven for 15 minutes, values approximating the accepted ones for moisture were obtained. This principle is also utilized by the Carter-Simon moisture tester, which is operated at 155°C. for 15 minutes (the time and temperature recommended by the manufacturer for drying starch). When the same time and temperature were used with the Carter-Simon moisture tester the results agreed with the moisture content established by the basic method (Table 4). It must be kept in mind, however, that rapid drying at higher temperatures gives only approximate values.

TABLE 6.—*Summary of loss in weight (per cent) of potato starch (Sample 1) dried to constant weight at various temperatures\* by different methods*

TEMPERATURE	TYPE OF OVEN					
	BRABENDER LOSS IN WT.	MECHANICAL CONVECTION LOSS IN WT.	CARTER-SIMON LOSS IN WT.	AIR LOSS IN WT.	DISTILLATION LOSS IN WT.	VACUUM LOSS IN WT.
°C.						
76					15.50	
80						16.48
100	16.00	16.00		15.61		16.57
110					16.20	
120	16.30					
135	16.40	16.53	16.24	16.58		
137					16.33	
146					16.40	
150	16.55	16.65		16.66		
155	16.70		16.52			
160	16.85					
170	16.95					
180		16.68				

\* Areas enclosed indicate the temperature range that may be used for determining the moisture content of potato starch by any given drying method.

Distillation methods for the determination of moisture gave results of the same order as those given by the oven-drying methods (Table 5). Generally, as the temperature of distillation was raised, the volume of water distilled from the sample increased. Because of their low boiling points, 76° and about 110°C., carbon tetrachloride and toluene gave results that were too low, as indicated by the steep part of the lower portion of the curve in Figure 3. Xylene and tetrachloroethane, whose boiling points would fall on the flat portion of the curve in Figure 3, gave percentages of moisture in agreement with those of the basic method.

TABLE 7.—*Moisture in potato starch (Sample 1) determined by Karl Fischer reagent*

RUN NO.	H <sub>2</sub> O
	<i>per cent</i>
1	16.14
2	16.01
3	16.17
4	16.04
5	16.00
6	16.08
7	16.00
Average	16.06

Owing to the low specific gravity of xylene, starch settles out when this solvent is used, and care should be taken to prevent charring on the bottom of the flask. In this experiment charring was avoided by slow distillation and intermittent shaking of the distilling flask. With tetrachloroethane, the distillation could be carried out more rapidly, since the starch floated on the surface of the liquid.

Table 6 gives a summary of the percentage losses in weight of potato starch dried to constant weight by different methods of various temperatures. The moisture values by the basic method and close approximations to it by other methods are indicated by enclosing lines.

The percentage of moisture in Sample 1, potato starch, determined by the Karl Fischer reagent is 16.06 per cent (Table 7), which is considerably lower than that obtained by the basic method (16.43 per cent), but higher than the values obtained by gravity-convection air oven drying at 100°C. (15.60 per cent).

Most of the data reported in the literature for the determination of moisture by the Karl Fischer reagent were compared with results obtained by drying in gravity-convection air ovens at approximately the boiling point of water (100°C.). They show, almost without exception, that the chemical method gives higher results. The conclusion drawn was that recovery of moisture by the chemical method is more nearly complete than



is recovery by oven-drying methods at or around the temperature of boiling water (6). However, if the results by the chemical method were compared with those obtained by oven drying in the range 135°–145°C., the recovery of moisture would be found to be less complete by the chemical method than by the oven-drying methods.

### SUMMARY

The effects of time and temperature on the loss in weight of potato starch by oven drying have been studied. From this study the conditions of drying that give the most nearly reproducible values for moisture content have been determined. By use of a Brabender moisture tester it was established that heating to constant weight at temperatures in the range of 135°–145°C. gives reproducible values. By using this basic procedure, it was found possible to modify several of the commonly used methods and obtain values in agreement with results established by the basic method. These methods have been found to be applicable to the determination of moisture in various agricultural products such as leafy vegetables and certain natural rubber-bearing plants, but experience indicates that the optimum conditions of time and temperature must be determined for each particular material.

### ACKNOWLEDGMENT

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## PERSISTENCE OF MONOCHLORACETIC ACID IN FRUIT JUICES AND CARBONATED BEVERAGES

By JOHN B. WILSON (Food Division,\* Food and Drug Administration, Federal Security Agency, Washington, D. C.)

Since it has been claimed that when monochloroacetic acid is added as a preservative to fruit juices and carbonated beverages the acid hydrolyzes and disappears as such within a short time, several experiments were conducted to establish whether or not hydrolysis does occur, and if so to what extent.

Creme Soda and Carbonated Orange Beverage were selected as representing two distinct types of carbonated beverages.

The Creme Soda Flavor was prepared using the formula given below.

0.24 gram vanillin  
0.10 gram coumarin  
0.1 ml. geraniol  
5 ml. lemon extract  
33 ml. alcohol to dissolve the above ingredients.

One gram of caramel flakes was dissolved in 40 ml. of water and mixed gradually with the alcoholic solution of the flavoring ingredients. Water was then added to complete a volume of 100 ml.

The Creme Soda Sirup was prepared by dissolving 18 lbs. of sugar in 1 gallon, 60 fl. ozs. of water, and adding 3 fl. ozs. of Creme Soda Flavor and 45 ml. of citric acid solution (100 grams in 200 ml. of solution).

The Orange Beverage Flavor was made using the formula given below.

6 grams gum arabic	18 grams glycerol
75 grams sugar	0.6 ml. sesquiterpeneless oil
3 ml. orange oil	100 ml. water

The ingredients were placed in a large beaker and mixed by hand with a stirring rod and finally with a power stirrer until homogeneous; 3 grams of tartrazine and 3 grams of ponceau were added as the stirring continued. The mixture was then poured into a 200 ml. volumetric flask, which was filled to the mark with water, and mixed thoroughly.

The Orange Beverage Sirup was then prepared as follows:

6.9 liter burred and strained orange juice	18.9 lbs. sugar
190 ml. Orange Soda Flavor	90 ml. citric acid solution (100 g. in 200 ml. of solution)

The whole was mixed thoroughly in a 5-gallon bottle. Each batch of sirup was divided into the required number of portions, the volume of each portion being governed by the number of bottles of beverage to be made, and monochloroacetic acid solution was added to give the desired concentration in the finished beverage. The sirups were then measured with gradu-

\* W. B. White, Chief.

ated cylinders into soda bottles, which were closed with cotton plugs, kept at 40°F. overnight, and put through the bottling machine the next morning at a commercial plant.

From the above formulas it was calculated that the beverages had a composition similar to the average soda of the type in question, as judged from Table 3, Sugar Contents Found in Carbonated Beverages, "A. B. C. B. Plant Operation Manual of Maintaining Quality and Uniformity" (page 7). The cream sodas contain about 11.2 per cent of solids and about 1 grain of citric acid per bottle. The orange beverages contain about 13.3 per cent solids, about 2 grains of acid, and about 10.2 per cent of orange juice.

The pressure was measured on occasional bottles of the beverages and found to average 2 volumes of gas for the cream soda and 1.8 volumes for

TABLE 1.—*Monochloracetic acid in stored carbonated beverages*

BEVERAGE	GRAMS MONOCHLORACETIC ACID/100 ML. BEVERAGE					
	CREME SODA			CARBONATED ORANGE BEVERAGE		
	B	H	S	BO	HO	SO
ADDED ACID	0.0010	0.026	0.260	0.0010	0.026	0.263
STORAGE						
<i>days</i>						
2	0.0004	0.026	0.248	0.0006	0.025	0.255
7	0.0010	0.025	0.244	0.0011	0.025	0.248
20	0.0004	0.026	0.250	0.0009	0.026	0.251
56	0.0011	0.025	0.250	0.0009	0.025	0.249
114	0.0005	0.026	0.248	0.0009	0.025	0.250
267	0.0006	0.024	0.236	0.0009	0.024	0.240
380	0.0009	0.026	0.236	0.001	0.024	0.239
580	0.0010	0.026	0.244	0.0010	0.025	0.229

the orange beverage, which were slightly lower than the averages found by the A. B. C. B., viz. 2.8 and 2.3 volumes of CO<sub>2</sub>, respectively.

The beverages were stored in the laboratory at room temperature. They were unprotected from the heat of the summer and were placed in such a position as to be exposed to sunlight on various occasions. Samples were opened after the periods of storage indicated in Table 1 and analyzed for monochloracetic acid. Beverages H, HO, S, and SO were analyzed by Method I given below, using 100 ml. of sample in the case of H and HO, and 25 ml. in the case of S and SO.

In the case of beverages coded B and BO (Table 1) the amount of monochloracetic acid was so small that only about 0.2 ml. of silver nitrate solution would have been used up by 100 ml. of sample. Therefore, in the interest of greater accuracy, 150 ml. of sample was extracted, and weaker solutions were used as described in Method II.

The data in Table 1 show that there is little if any loss of added mono-

TABLE 2.—*Monochloroacetic acid in stored bottled apple juice*

PERIOD OF STORAGE	MONOCHLORACETIC ACID (GRAMS/100 ML. JUICE)		
	ADDED	FOUND PASTEURIZED	FOUND UNPASTEURIZED
<i>days</i>			
81	0.010	0.009	0.010
215	0.010	0.009	
227	0.010		0.010 0.010 0.009
392		0.010	0.010 0.010
81	0.031	0.026	0.027
212	0.031 0.031		0.029 0.029
215	0.031	0.027	
224	0.031		0.028 0.029
392		0.027	0.028
81	0.052	0.049	0.048
215	0.052	0.048	
222	0.052 0.052		0.048 0.048
224	0.052		0.048 0.048
392		0.047	0.047
248	0.104		0.091
250			0.091
392			0.096 0.089 0.095 0.092

chloroacetic acid in carbonated beverages stored for periods exceeding a year and a half.

Packs of apple juice were prepared in which both pasteurized and unpasteurized juices were treated with monochloroacetic acid at several levels and analyzed after extended storage by Method I, using 100 ml. of sample for each determination. In the case of the pasteurized juice the proper

quantities of monochloracetic acid solution were measured with a pipet into bottles as they left the warming machine, after which they were filled with hot juice and capped in the usual manner. In the case of the unpasteurized juice the monochloracetic acid solution was mixed with juice from the pressing machine in a large container and filled by hand into the bottles. The determinations of monochloracetic acid are given in Table 2.

The data in Table 2 show little, if any, loss of monochloracetic acid in bottled apple juice stored for over a year at room temperature.

In preparing the pack of orange juice, the desired quantity of monochloracetic acid solution was measured into a 5-gallon bottle, and several gallons of freshly pressed orange juice were added and mixed. The juice was then filled into 10 fluid ounce cans and sealed without processing. The cans of orange juice containing 0.010 gram of monochloracetic acid per 100 ml. "blew up" before the first analysis was made. Those containing other levels of monochloracetic acid were analyzed on three occasions. The cans were stored at about 40°F. The results are given in Table 3.

TABLE 3.—*Monochloracetic acid in stored canned orange juice*

PERIOD OF STORAGE	MONOCHLORACETIC ACID (GRAMS/100 ML JUICE)	
	ADDED	FOUND
<i>days</i>	0.010	Lost
70	0.030	0.027
220	0.030	0.029
384	0.030	0.029
70	0.050	0.047
220	0.050	0.047
384	0.050	0.045
70	0.100	0.088
220	0.100	0.094
384	0.100	0.092

These data show that no loss occurs in the monochloracetic acid content of canned orange juice on storage for over a year.

An additional storage experiment was carried out on commercial orange juice and grapefruit juice containing monochloracetic acid. These packs were produced by a manufacturer experimenting with monochloracetic acid as a preservative. The quantity of monochloracetic acid added by the manufacturer is not known, but the product was analyzed soon after receipt and again after three intervals of storage at about 40°F. The sweetened juices were from the same batch as the unsweetened, with dextrose added so as to raise the orange juice from 9° to 14° Brix and the grapefruit juice from 8.6° to 12.5° Brix. The results of analysis are given in Table 4.

TABLE 4.—*Monochloracetic acid in stored canned citrus juices*

FRUIT JUICE		MONOCHLORACETIC ACID (GRAMS/100 ML. JUICE)			
CAN	STORAGE	ORANGE		GRAPEFRUIT	
		SWEETENED	UNSWEETENED	SWEETENED	UNSWEETENED
	<i>months</i>				
A	1 (about)	0.037	0.039	0.070	0.074
B		0.037	0.040	0.070	0.074
C	13	0.036	0.038	0.067	0.067
D		0.035	0.038	0.068	0.074
E	22	0.036	0.038	0.071	0.074
F	30	0.037	0.036	0.072	0.071
G		0.036	0.035	0.071	0.071

The results in Table 4 show that there is no essential change in the monochloracetic acid content even after about two and one-half years of storage.

The methods of analysis used were based on the work reported by the writer in a former publication<sup>1</sup> and are given below.

#### I. DETERMINATION OF MONOCHLORACETIC ACID

(Applicable to carbonated beverages and fruit juices containing 5–150 mg  $\text{CH}_2\text{Cl COOH}$  in 150 ml.)

##### APPARATUS

*Continuous extractor.*<sup>2</sup>—Make the outer part 45 cm. long from 43 mm. tubing with side tube issuing 25 cm. above the bottom, and fitted with standard taper, drip tip, joint 24/40. Make the inner tube 40 cm. long from 12 mm. tubing. Use a 250 ml. conical flask with suitable joint to hold the overflowing ether extract.

##### REAGENTS

*Silver nitrate solution.*—(1 ml. =  $\pm 5$  mg  $\text{CH}_2\text{Cl COOH}$ ) Dissolve 9 grams of  $\text{AgNO}_3$  in water and dilute to 1 liter.

*Ammonium sulfocyanate solution.*—(1 ml. =  $\pm 5$  mg.  $\text{CH}_2\text{Cl COOH}$ .) Dissolve 4.03 grams of  $\text{NH}_4\text{CNS}$  in water and dilute to 1 liter. Standardize against pure  $\text{NaCl}$  solution, 3.093 grams per liter, which contains 1.8762 grams of  $\text{Cl}$  (equivalent to 5 grams of monochloracetic acid, which contains 1.8764 grams of  $\text{Cl}$ ).

*Ferric indicator.*—A saturated solution of ferric ammonium alum.

##### DETERMINATION

In the outer part of the continuous extractor, place such a quantity (not over 150 ml.) of carbonated beverage, apple juice, grapefruit or orange juice as contains 5–100 mg. of  $\text{CH}_2\text{Cl COOH}$ . Dilute if necessary to 150 ml., add 3–5 ml. of  $\text{H}_2\text{SO}_4$ , mix, and extract with ether 2–3 hours. Tilt the extractor in such a way as to drain as much as possible of the ether into the flask; disconnect the flask, add 25 ml.

<sup>1</sup> *This Journal*, 25, 145 (1942).

<sup>2</sup> *Methods of Analysis*, A.O.A.C., 1940, Fig. 57-B, p. 594.

1 N NaOH in excess of that required to make the water layer alkaline to litmus paper after shaking, shake, and evaporate the ether on the steam bath until only about 25 ml. of liquid remains, hastening the process by passing a current of air into the mouth of the flask. Digest on the steam bath for 2 hours or boil under a reflux condenser for  $\frac{1}{2}$  hour. Add 50 ml. of water, 15 ml. of  $\text{HNO}_3$ , and a known volume of the  $\text{AgNO}_3$  solution, in excess. Shake  $\frac{1}{2}$ –1 minute, add the ferric indicator, and titrate the excess Ag with the  $\text{NH}_4\text{CNS}$  solution. In the same way, titrate a quantity of  $\text{AgNO}_3$  solution equal to that added to the sample. The difference between the two titrations is a measure of the  $\text{CH}_2\text{Cl COOH}$ .

## II. DETERMINATION OF MONOCHLORACETIC ACID

(Applicable to carbonated beverages and fruit juices containing from 1 to 10 mg.  $\text{CH}_2\text{Cl COOH}$  in 150 ml.)

### REAGENTS

*Dilute silver nitrate solution.*—Pipet 20 ml. of the  $\text{AgNO}_3$  solution (1 ml. =  $\pm 5$  mg.  $\text{CH}_2\text{Cl COOH}$ ) into a 100 ml. volumetric flask and fill to the mark with water.

*Dilute sulfocyanate solution.*—Pipet 20 ml. of the  $\text{NH}_4\text{CNS}$  solution (1 ml. =  $\pm 5$  mg.  $\text{CH}_2\text{Cl COOH}$ ) into a 100 ml. volumetric flask and fill to the mark with water. Standardize the dilute sulfocyanate solution against some of the  $\text{NaCl}$  solution diluted 20 ml. to 100 ml. to obtain the exact strength of the sulfocyanate solution.

### DETERMINATION

Extract and hydrolyze the monochloracetic acid as directed for samples containing more than 5 mg. per 150 ml.; add 50 ml. water, 15–20 ml. of  $\text{HNO}_3$  and 1 ml. of the ferric indicator. At the same time prepare a blank containing 75 ml. water, 15 ml.  $\text{HNO}_3$ , and 1 ml. of the ferric indicator. To each add 5 ml. of the dilute  $\text{AgNO}_3$  solution and mix thoroughly. To the blank add 4 ml. of the dilute sulfocyanate solution, and to the determination gradually add small quantities of the same solution until the pink color fades slowly on mixing. Shake both solutions and filter. Add about 50 ml. of water to each flask when empty and pour into the respective filter when it is empty. When all the wash water has passed through, complete the titrations, matching the color of the determination to that of the blank. In this case each ml. difference in titration =  $\pm 1$  mg.  $\text{CH}_2\text{Cl COOH}$ .

### CONCLUSIONS

Little, if any, loss of monochloracetic acid was found to occur:

- (1) When fruit-type and non-fruit-type carbonated beverages containing this preservative were stored over a period of 19 months.
  - (2) When pasteurized or unpasteurized apple juice containing added monochloracetic acid was bottled and stored for 13 months.
  - (3) When canned orange juice and grapefruit juice containing monochloracetic acid were stored for 30 months.
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## DETERMINATION OF PEEL OIL IN CITRUS JUICES

By JOHN B. WILSON (Food Division\*, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

In February, 1941, W. C. Scott of the Fruit and Vegetable Products Laboratory, Weslaco, Texas, published a paper on "Determination of Peel Oil in Grapefruit Juice."<sup>1</sup> He recommended the steam distillation of 3 liters of grapefruit juice, using as a receiver a Bromwell fusel oil separatory funnel, or as an alternate, a Wilson receiving flask<sup>2</sup>. The method is described in "United States Standards for Grades of Canned Grapefruit Juice," effective December 15, 1941, of the U. S. Marketing Service. That agency uses the Wilson flask as a receiver.

Some manufacturers use an oil separatory trap for oils lighter than water.<sup>3</sup> As an economy measure they have also cut down the volume of the sample to 1 liter or even less. Several control chemists have designed apparatus for their own use in determining peel oil in citrus juices.

The writer has used both the steam distillation procedure, which involves measurement of the oil in a Wilson flask, and the refluxing procedure, in which the oil is measured by means of the oil separatory trap. Commercially manufactured specimens of these two types of apparatus have dimensions which give greater accuracy of measurement to the Wilson flask, since the 0.1 ml. graduations are 3.6 mm. apart, while in the oil separatory trap they are 1.7 mm. apart. In the latter apparatus quantities of oil in excess of 0.5 ml. tend to separate into two or more portions, one or more of which frequently move down the tube and find their way back to the boiling flask. If the distillation is stopped when this occurs the reading will be in error. The tendency to separate increases when the diameter of the graduated portion of the tube is lessened in the interest of greater accuracy of reading.

The writer has remodeled the oil separatory trap (see Figure 1) in a manner which overcomes the several objections given above to make it more suitable for the accurate determination of peel oil in citrus juices. The salient features of the new model are: (1) the graduated portion of the tube consists of a 2 ml. section of a 5 ml. Mohr pipet, in which the spaces occupied by each 0.1 ml. of liquid average 4 mm.; (2) the reflux is arranged so that it does not drip directly upon the oil in the tube but falls upon an incline, whence it runs into the graduated portion of the tube; and (3) a bulb is blown immediately below the graduated portion of the tube. The length of the space between graduations increases the accuracy of the reading. The displacement of the drip from the reflux largely pre-

\* W. B. White, Chief.

<sup>1</sup> *This Journal*, 24, 165 (1943).

<sup>2</sup> *Methods of Analysis*, A.O.A.C., 1940, 331.

<sup>3</sup> *Ibid.*, 471.





When the modified oil distillation trap is used, the sample may consist of as little as 1 liter of citrus fruit juice placed in a 2 liter boiling flask, to which the trap is attached after being filled as far as possible with distilled water. A reflux condenser is then attached to the trap. Boiling for 30 to 45 minutes was found to be suitable for the determination.

When orange oil in synthetic solutions was determined by the steam distillation method, a larger percentage of oil was obtained when a 3 liter sample was used than when a 1 liter sample was used. Table 1 contains results obtained when these two quantities of sample were steam distilled.

TABLE 1.—*Recovery of orange oil by the steam distillation method*

OIL ADDED	SAMPLE USED	OIL FOUND		
		3 LITERS	1 LITER	
<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>loss (ml.)</i>
0.100		0.095	0.090	0.10
		0.092		
0.067		0.058	0.055	0.12
			0.053	0.07
0.050		0.045	0.040	0.10
			0.045	0.05
0.040		0.038	0.033	0.05
			0.029	0.11
0.035		0.033	0.030	0.05
0.030		0.027	0.025	0.05
			0.022	0.08
Av. loss (ml.)		0.05		0.08
Av. loss (% oil)		0.0017		0.008

The data in Table 1 show that losses are often greater with the smaller sample. For greatest accuracy, therefore, a method for recoverable oil

TABLE 2.—*Recovery of citrus oil by modified oil distillation trap*

ADDED	FOUND								
	ORANGE OIL			GRAPEFRUIT OIL			LEMON OIL		
<i>per cent</i>	<i>per cent</i>	<i>loss (ml.)</i>	<i>Cor *</i>	<i>per cent</i>	<i>loss (ml.)</i>	<i>Cor.*</i>	<i>per cent</i>	<i>loss (ml.)</i>	<i>Cor *</i>
0.100	0.090	0.10	0.095	0.090	0.10	0.097	0.090	0.10	0.095
0.075	0.069	0.06	0.074	0.067	0.08	0.074	0.068	0.07	0.073
0.060	0.055	0.05	0.060	0.049	0.11	0.056	0.050	0.10	0.055
0.050	0.046	0.04	0.051	0.042	0.08	0.049	0.044	0.06	0.049
0.040	0.035	0.05	0.040	0.032	0.03	0.039	0.035	0.05	0.040
0.030	0.027	0.03	0.032	0.025	0.05	0.032	0.027	0.03	0.032
0.025	0.022	0.03	0.027	0.020	0.05	0.027	0.020	0.05	0.025
0.020	0.015	0.05	0.020	0.013	0.07	0.020	0.018	0.02	0.023
0.015	0.012	0.03	0.017	0.010	0.05	0.017	0.012	0.03	0.017
0.010	0.005	0.05	0.010	0.005	0.05	0.012	0.008	0.02	0.013
Av. loss		0.05			0.07			0.05	

\* Corrected for average loss.

should specify the volume of sample to be used, and analysts should adhere to the quantity of sample specified.

A series of determinations of citrus oils in synthetic solutions was made using the modified oil distillation trap. The sample consisted of 1 liter of distilled water to which had been added quantities of orange, grapefruit, and lemon oils which cover the spread likely to be found in fruit juices. These results are given in Table 2.

The data in Table 2 indicate that when the recovered quantity of oil is corrected by adding the average loss for the particular kind of oil, 23 of 30 results are within 0.002 per cent of the percentage of added essential oil. Of the remaining seven determinations, three contained 0.1 per cent of oil, which is so high as to be seldom if ever met with in commercial fruit juices.

#### SUMMARY

A modified apparatus for the determination of peel oil in citrus fruit juices is presented, having the following advantages:

- (1) Accurate results are possible with a 1 liter sample.
- (2) Constant attention is not required during the determination.
- (3) The distilled oil remains segregated and is not broken up into droplets during the determination, and thus results can be read off at once after its completion.

Data on peel oil recovery are presented using this apparatus.

Results obtained by steam distilling 3 liter and 1 liter sample are compared.

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#### DETECTION OF DECOMPOSITION IN LIQUID, FROZEN, AND DRIED EGGS\*

By HENRY A. LEPPER, M. T. BARTRAM and FRED HILLIG  
(U. S. Food and Drug Administration, Washington, D.C.†)

One of the most valuable foods nutritionally, the highly prized egg, was among the first to be chosen for shipment to the Allied nations in the current war. Its fragility and perishability destined its shipment in shell form to failure at the outset, and frozen eggs offered little advantage over shell eggs because of the lack of sufficient refrigerated cargo space. The choice of drying as the process of preparation was therefore inevitable as best suited to meet the situation. The resultant saving of shipping space was an important factor.

During 1942 purchases by the Government exceeded two hundred million pounds of dried whole eggs, although previously production of

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† Contribution from the Food Division, W. B. White, Chief; Division of Bacteriology, A. C. Hunter, Chief.

this commodity in the United States had been negligible. This rapid expansion of the industry was accomplished by enlargement of existing facilities, conversion of dried milk plants, and construction of new equipment. In many instances manufacturers entered the field with little or no knowledge of the fundamental requirements for the production of an acceptable, wholesome product, and many of them also lacked the necessary equipment. The preparation of liquid eggs for drying is the same as for freezing. Additional refrigeration is necessary to preserve the accumulating liquid eggs while they are being held for drying, and members of the egg trade who added the production of dried egg to their operations often did not have such refrigeration facilities. Among these newcomers there were unfortunately a few who were attracted only by the promise of substantial profit, but who seemed totally indifferent to their obligation to produce an acceptable article of food. It is therefore not surprising that dried whole eggs which were unacceptable under Government purchase specifications were delivered. Since the purchasing agency had no authority over the disposition of the rejected lots, it was possible for the trade to mix them with good eggs and resubmit them to the Government. They might also be diverted to civilian channels for use in manufactured foods. That food manufacturers accepted such eggs during a period of acute scarcity, especially when they could be concealed in manufactured foods, was not surprising. Lack of experience with authentic samples prepared from good eggs and decomposed eggs handicapped analytical interpretations, and furthermore the earlier methods employed to establish decomposition in dried eggs (e.g. the acidity of ether extract) were found to be inadequate. The regulatory food official was thus confronted with a serious problem in law enforcement. Accordingly an investigation was undertaken to obtain fundamental data applicable to its solution.

#### PREPARATION OF AUTHENTIC BATCHES OF DRIED EGGS

Through the cooperation of an establishment in Chicago the usual commercial equipment for breaking-out various grades and types of eggs was made available. Shell eggs were chilled to 40°F., candled and segregated, individually broken, visually inspected and smelled. In each experiment sufficient liquid egg to produce about 25 pounds of dried egg was prepared within 30 minutes. After thorough mixing and chilling in a cold room to between 40° and 48°F., samples were drawn from the batch and sent to the Chicago laboratory of the Food and Drug Administration. Bacteriological and chemical analyses were begun within 30 minutes, and the temperature of the samples did not rise more than a few degrees. The odor of the liquid eggs was noted and recorded. Two samples of from two to three pounds each from each batch were placed in tin cans and frozen in a sharp freezer. The batches of liquid egg were then transported in closed 10 gallon milk cans to a small, commercial continuous spray-type drier with a

capacity of 50 pounds of dried egg per hour. In no batch was there any change in odor during approximately the one hour between preparation and introduction into the drier. The temperature rise of the liquid egg during this interval did not exceed 2°F. The dried eggs were packed in 30 pound tin cans of the type used for frozen eggs and shipped to Washington, D. C. for taste test and analysis. The frozen-egg samples, after being held six months in the freezer, were packed in dry ice and shipped to Washington, D. C. They arrived in a hard-frozen condition and were so held until analyzed. The experimental batches consisted of three groups: (1) good eggs; (2) liquid eggs held unrefrigerated with delayed drying; and (3) eggs mixed with known amounts of inedible eggs.

In the good egg group, twelve batches of liquid eggs were prepared. The eggs used were from current-receipt stocks of the plant at which the work was being done. During the candling, the eggs were segregated into two grades, one better than the other as judged by the size of the air cell and the mobility and visibility of the yolk. The selection was verified by the physical condition of the egg after breaking. Two batches of each grade were dried promptly after preparation. One batch of the better grade was held 22 hours and one of the lower grade 20 hours at 35°F. to test the effect of holding for about a day at low temperature. One batch of the lower grade was held six hours at 85°F. to note the effect of higher temperature over a relatively short holding period. The influence of freezing and thawing was tested on two batches from ungraded current receipts. The liquid eggs were frozen at -5°F. in conventional 30 pound egg cans. After several days, one batch was allowed to stand at about 70°F. for 24 hours, the other for 48 hours. At the end of 24 hours the former can still contained considerable hard-frozen egg that had to be broken up before preparation of a liquid mix. At the end of 48 hours the other can still contained some unmelted egg. One batch prepared from edible eggs was heated to 510°F. during the drying, a temperature much higher than that employed by the industry, to determine what effect overheating or scorching might have on the constituents being studied.

"Off" odor and flavor in eggs had often been attributed by members of the industry to the feed of the hens. To test this theory eggs from hens under controlled diets were obtained through the cooperation of the Bureau of Animal Industry of the United States Department of Agriculture. Hens in four groups of four pens each received a typical all-mash laying diet. One group was used as a control. To the diet of one group very large quantities of both fish oil and fish meal were added. Linseed and cottonseed meals, respectively, were included in the diet of the other two groups. Upon breaking-out no foreign odors were observed in any of the eggs from these feeding experiments. Several eggs in each lot were also tasted, and no foreign taste was noted.

For the second group, that in which eggs were held in the liquid state

TABLE 1.—*Authentic egg batches*

SAMPLE NO.	DESCRIPTION	ODOR LIQUID EGGS
Group 1—Good Eggs		
A1	Better grade current receipts	None
A2	Better grade current receipts	None
A3	Better grade current receipts held 22 hours at 35° F.	None
B1	Lower grade current receipts	None
B2	Lower grade current receipts	None
B3	Lower grade current receipts held 20 hours at 35° F.	None
B4	Lower grade current receipts held 6 hours at 85° F.	Off*
RF1	Current receipts frozen and thawed for 24 hours at 70° F.	None
RF2	Current receipts frozen and thawed for 48 hours at 70° F.	None
F1	Eggs from hens fed regular diet (Control)	None
F2	Eggs from hens fed with added fish meal and fish oil	None
F3	Eggs from hens fed with added linseed meal	None
F4	Eggs from hens fed with added cottonseed meal	None
RII	Current receipt eggs overheated by drying at 510° F.	None
Group 2—Eggs Held at 85° F.		
B5	Lower grade current receipts held 18 hours	Sour
RO2	Lower grade current receipts with 2.5% added odorless addled eggs held 22 hours	Sour
RO3	Lower grade current receipts with 5% added odorless addled eggs held 18 hours	Sour
RR2	Lower grade current receipts with 2.5% mixed rots held 23 hours	Sour
RR3	Lower grade current receipts inoculated with mixed rots	Sour

\* "Off" is applied to a detectable odor not definitely assignable to decomposition.

unrefrigerated for a delayed period before drying, the lower grade of current-receipt eggs was used, and five batches were prepared. One batch consisted of good eggs. To the other four definitely inedible eggs were added before the holding period. One batch contained 2.5 per cent, another 5 per cent by weight of odorless addled eggs, and the third contained 2.5 per cent by weight of mixed rots. The term "odorless addled eggs" is here applied to eggs which on breaking show a ruptured yolk membrane with the yolk more or less dispersed into the white, but with no odor. Those having an abnormal odor were classed as mixed rots. Odorless addled eggs are often high in bacteria and doubtless would become mixed rots if left unbroken for a day or so longer. These added inedible eggs were collected from the available supplies during the selection of the edible eggs used in the batches, and were added only after agreement among those engaged in the preparation of the various batches as to their classification. The last batch was inoculated with mixed rots which had been rejected by the employees of the plant in the routine of production going on concurrently with these experiments. The prevailing

temperature of the atmosphere during the holding period was 85°F. No attempt was made to determine the effect of other temperatures as this temperature is within the range prevailing during most of the season when eggs are customarily broken-out in plants over the country. The time of holding was from 18 to 23 hours, and extended from the preparation one day until the next morning when the mixture was sampled and sent to the drier. The periods were chosen for convenience and not to determine the length of exposure necessary to cause decomposition detectable by odor in the liquid egg. In some instances a souring was evident in about six hours. A summary of the identity of the batches in the good and

TABLE 2.—*Authentic egg batches*

SAMPLE NO.	DESCRIPTION	ODOR LIQUID EGGS
Group 3—Added Inedible Eggs		
RO1	Current receipt egg with 5% odorless addled eggs	None
RR1	Current receipt egg with 5% mixed rots	None
RM	Current receipt egg with 10% moldy	Moldy
RB	Current receipt egg with 0.3% black rots	Putrid
RU	Current receipt egg with 2% musty	Musty
BL	Weak yolk and blood ring eggs	None

held egg groups together with the odor of the liquid egg is given in Table 1.

The third group of batches was prepared under conditions simulating manufacturing practices so careless as to include inedible eggs. In such batches the amounts of inedible eggs indicated in Table 2 were added to current-receipt eggs and all were promptly dried.

The odorless addled and the mixed-rot eggs have been described. The moldy eggs had mold growing within the egg, often traceable to a crack in the shell. Usually they had a moldy odor. The black rots were of the type well known under this name in the trade. Such eggs are in an advanced state of decomposition and are readily recognized on candling by their gray or black color. They have a striking, putrid odor. Only in exceptional cases would they find their way into commercial egg products. The musty eggs were those having a pronounced musty odor although their appearance was that of a sound egg when broken from the shell. This musty condition has been shown to be due to the development of either of two species of the genus *pseudomonas* (1), which sometimes causes the white to become cloudy and greenish. Musty eggs are regarded by the trade as inedible and unfit for use even before the development of visual changes in the white. Even one musty egg may be sufficient to impart characteristic odor to a large batch of liquid egg and the industry

is particularly anxious to keep them out. Another batch consisted of weak-yolk eggs with a small number having blood rings. Weak-yolk eggs have a very tremulous yolk, which often ruptures when the contents of the shell are dropped into the breaking cup. They have no odor. Blood rings are eggs having an enlarged germinal spot surrounded by a complete or partial ring of blood. Such eggs are universally regarded as inedible.

#### METHODS OF ANALYSIS

The methods chosen for the examination of the liquid eggs at Chicago and later of the dried and frozen eggs at Washington were those which showed promise in the light of previous experience or literature articles. The bacteriological examinations consisted of a count of viable bacteria, total bacteria by means of microscopic count, and a determination of members of the coliform group. The viable and coliform bacteria were determined by the methods adopted by the A.O.A.C. (2). The nutrient agar plates were counted after 72 hours incubation at 32°C. The microscopic count was made by the method of the American Public Health Association (3), modified for dried eggs by the use of a 2 sq. cm. area instead of 1 sq. cm., and for liquid and frozen eggs by spreading 0.01 ml. of the undiluted material over areas of 1 and 2 sq. cm., respectively.

Numerous chemical methods of examination have been proposed from time to time for demonstrating decomposition in eggs. Acidity of ether extract has been so used. An official method and a rapid tentative method have been adopted for this determination by the A.O.A.C. (4). A tentative method for ammonia nitrogen in liquid eggs has also been adopted (5). The influence of decomposition on the partition of phosphates into the organic and inorganic forms has initiated methods for the determination of phosphate in eggs (6) (7) (8). None of the proposed phosphate methods has been recognized by adoption by the Association. A new "decomposition quotient" based on the ratio of phosphate insoluble in isopropyl alcohol to that soluble in aqueous alcohol solution has been suggested as valuable in demonstrating cheesy decomposition in eggs (9). Accurate methods for the determination of volatile acids have been proposed (10) and developed (11). They have already been found of value in confirming decomposition in fish products (12) (13) (14). A preliminary report (15) has also indicated their value with eggs. A method for lactic acid has been developed and applied to dairy (16) (17), tomato (18), and fruit (19) products. Perhaps because of the ease of determination the sugar content of eggs has been given attention in a number of investigations on decomposition.

The following chemical determinations were made on the samples of the liquid, frozen, and dried eggs: Acidity of ether extract by the rapid method; volatile-base nitrogen by a revised procedure (in lieu of the ammonia nitrogen method); decomposition quotient; volatile and lactic



acids (by the methods given below); reducing sugars by the A.O.A.C. method (20); total solids; and pH. The frozen eggs were examined by the "drill and smell" test just before analysis. At the time of analysis of the dried eggs taste tests were made on 10 gram samples as follows: The samples were mixed with three times their weight of water and the mixture, continuously stirred, was heated by immersion of the container in gently boiling water until the egg coagulated. The scrambled egg was then tasted. As will be shown later the methods for volatile and lactic acids were found to be extremely useful. The details of these methods as adapted to eggs follow:

#### LACTIC ACID IN EGG PRODUCTS

##### PREPARATION OF SAMPLE

*Liquid or Frozen Eggs.*—Transfer 20 grams to a tared 200 ml. Erlenmeyer flask, add approximately 30 ml. of water, and shake thoroughly. Add 10 ml. of normal  $\text{H}_2\text{SO}_4$  and 15 ml. of a 20% phosphotungstic acid, make to 125 grams with water, shake for about 1 minute, and filter through a folded filter paper. (50 ml. of filtrate will be found to weigh 50 grams.)

*Dried Eggs.*—Mix 5 grams and 50 ml. of water into a uniform paste with the aid of a stirring rod and add with constant stirring 10 ml. of normal  $\text{H}_2\text{SO}_4$ , followed by 12 ml. of a 20% phosphotungstic acid solution. Transfer the mixtures with water into a tared 200 ml. Erlenmeyer flask, and make to 125 grams with water. Shake for about 1 minute and filter through a folded filter paper. (50 ml. of filtrate will be found to weigh 50 grams.)

##### REAGENTS

*Standard barium lactate solution.*—Dissolve in about 10 ml. of water a quantity of a pure lactic acid salt, such as lithium, zinc, or calcium lactate, that will contain the equivalent of about 300 mg. of free lactic acid. Transfer the material to the extractor (for description of extractor see *This Journal* 25, 256 (1942), add 0.5 ml. of  $\text{H}_2\text{SO}_4$  (1+1), and adjust the volume to 50 ml. Extract with ether for 2 hours. Add about 20 ml. of water to the extraction flask, evaporate the ether on the steam bath, and carefully titrate the contents of the flask with 0.1 N  $\text{Ba}(\text{OH})_2$ . Transfer the neutralized material to a 200 ml. volumetric flask, make to mark, and shake. Pipet into a 500 ml. volumetric flask such a quantity of this barium lactate solution as will contain the equivalent of exactly 250 mg. of free lactic acid, make to mark, shake, and designate as *standard lactate solution*.<sup>1</sup> (2 ml. of this solution will contain the equivalent of 1 mg. of lactic acid.) Transfer 20 ml. of the standard lactate solution to a 100 ml. volumetric flask, make to mark, and designate as *dilute standard lactate solution*. (10 ml. of this solution will contain the equivalent of 1 mg. of lactic acid.)

*Carbon.*—To 10 grams of a high-grade carbon (Nuchar W is suitable, and Suchar, Darko G60, and Carbox E can also be used) in a 600 ml. beaker, add about 200 ml. of water and 30 ml. of normal HCl, and place on the steam bath for 20 minutes. Agitate continuously with air passed through cotton. Filter on a Büchner funnel and suck as dry as possible, tamping with a flattened rod. Transfer the cake to the beaker, add about 200 ml. of water, mix thoroughly, and refilter. Repeat the washing and filtering twice and dry in the water oven.

*Ferric chloride solution.*—Dissolve 2 grams of  $\text{FeCl}_3$  (analytical reagent) in water, add 5 ml. of normal HCl, and dilute to 200 ml.

<sup>1</sup> The standard lactate solutions for plotting the standard curve must be freshly prepared and promptly used.

## PLOTTING STANDARD CURVE

Transfer from buret to volumetric flasks graduated at 50 and 55 ml. the quantities of standard solutions listed in the left-hand column of Table A. In the right-hand column of Table A are given the quantities of lactic acid (mg.) that will be contained in the 40 ml. of filtrate obtained from each sample after the carbon treatment below, and that will therefore be read in the photometer. A blank using 40 ml. of water in place of the designated quantities of lactate solution must be included in the series.

TABLE A

SOLUTION TO BE TRANSFERRED TO 50-55 ML. VOLUMETRIC FLASK	LACTIC ACID IN 40 ML. ALIQOUT OUT OF 55 ML.
Dilute standard lactate solution	
ml.	ml.
6.90	0.5
13.80	1.0
27.60	2.0
Standard lactate solution	
8.25	3.0
11.00	4.0
13.75	5.0
16.50	6.0
19.25	7.0
22.00	8.0
24.75	9.0
27.50	10.0
30.25	11.0
33.00	12.0

To each flask add 6.6 ml. of 0.1 *N* HCl and water until the volume is about 40 ml. Now add 200 mg. of the prepared carbon, shake, and place on the steam bath for 10 minutes, mixing at frequent intervals. Cool, make to 55 mark with water, and as soon as possible filter through quantitative paper, pouring back until bright.

Transfer 40 ml. of each clear filtrate to a 50 ml. Nessler tube. As the 40 ml. of filtrate used contains only 4.8 ml. of the acid added during the carbon treatment, add 1.2 ml. of the HCl. (A total of 6 ml. of 0.1 *N* HCl is required in the tube.) Place each tube in a jacket of black paper. With one tube at a time, add 5 ml. of the FeCl<sub>3</sub> solution by pipet, make to mark, and mix. Pour the solution into a 4 inch photometer cell (preferably Pyrex) with plane parallel fused ends, the side walls of which are painted black, and read in neutral wedge photometer, using filter No. 46.<sup>2,3</sup> From the readings obtained, prepare a standard curve, plotting mg. of lactic acid as abscissae and scale readings as ordinates. Large-scale graph paper is recommended in order that more accurate interpolations may be made.

It is not necessary to prepare a new standard curve when a new batch of carbon or solution of FeCl<sub>3</sub> is used. However, a blank determination with water should be

<sup>2</sup> Clifford and Brice, *Ind. Eng. Chem., Anal. Ed.*, 12, 218 (1940). Other types of photometers can be used. Also, color comparisons can be made directly in the Nessler tube by customary procedure. See *This Journal*, 20, 135 (1937).

<sup>3</sup> On exposure to direct light the color fades, but protected as provided it is found to be stable a number of hours.

made and if this blank does not coincide with the original blank, readings can be brought into conformity with the curve by simply adding to or subtracting from the readings the observed amount of variance of the new blank from the old blank.

#### EXTRACTION<sup>4</sup>

Place 50 ml. of the filtrate obtained from the prepared sample and 0.5 ml. of  $\text{H}_2\text{SO}_4$  (1+1) in the inner tube of the extractor and connect to the longest bulb-type condenser available having an outlet not less than 1/2 inch inside diameter to obviate regurgitation of the ether. Run water through the condenser in sufficient quantity to obtain maximum condensation efficiency. Connect the extraction flask containing 200 ml. of ether, and lower the flask onto a hot plate that has been previously heated in order to prevent super-heating of the ether. Protect the extractor from the heat of the hot plate by an upright sheet of asbestos and extract until all the lactic acid is extracted. When the ether in the extraction flask is kept at rapid boiling and the condensing water is sufficiently cold to allow the condensed ether to return to the extraction flask in a steady stream, a 3-hour extraction period will deliver all the lactic acid. When this rate of extraction cannot be maintained because of high temperature of the water passing through the condenser, continue the extraction until the equivalent of 7500 ml. of ether has passed through the solution being extracted. The time required, "T," established for each set of new conditions, is calculated from 2 factors, "A," the quantity of ether necessary to fill the extractor to overflowing at the side-arm, which is a constant for each apparatus; and "B," the time in minutes required for quantity "A" to pass from the extraction flask and to fill the extractor. To determine "A," place 50 ml. of water and 0.5 ml. of  $\text{H}_2\text{SO}_4$  (1+1) in the extractor. With the extractor held upright, carefully pour ether into the inner tube until it just starts passing out of the side-arm. Determine "B" in the ordinary course of starting each determination. With a stop-watch, record the interval from the time the ether first drops from the condenser and falls into the inner tube to the time the first drops return to the extraction flask from the overflow into the side-arm. The time "T" necessary for 7500 ml. to pass through the apparatus equals  $7500 \text{ "B" / "A"}$ . The calculated "T" holds true only if the rate of boiling and condensing is unchanged throughout the extraction period.

#### DETERMINATION

To the flask containing the ether extract add 20 ml. of water and expel the ether on the steam bath. Do not allow the flask to remain on the steam bath after the ether has been expelled. Neutralize the contents of the flask with saturated  $\text{Ba}(\text{OH})_2$  solution (phenolphthalein indicator). Transfer to a 110 ml. volumetric flask with alcohol until the volume is about 90 ml. Heat almost to boiling on the steam bath, cool, make to mark with alcohol, and filter through a quantitative paper. To expel the alcohol, evaporate 100 ml. of the filtrate to about 10 ml., add about 50 ml. of water, and again evaporate to about 10 ml. (or the 100 ml. of filtrate may be evaporated to dryness on the steam bath). Add from a buret 6.6 ml. of 0.1 *N*  $\text{HCl}$  and transfer the contents of the beaker with water to a 50–55 ml. volumetric flask until the volume is about 40 mg. Add 200 mg. of the acid-treated carbon, immediately shake, and place on the steam bath for 10 minutes, mixing at frequent intervals. Cool, make to mark with water, and filter through a quantitative paper, pouring back until bright.

Transfer 40 ml. of the filtrate to a Nessler tube. (A total of 6 ml. of 0.1 *N*  $\text{HCl}$

<sup>4</sup> The procedures for the extraction and determination of lactic acid are those given in *This Journal*, 25, 355 (1942), except for a few editorial changes. In the previous directions lithium lactate was used as a standard, but it has been replaced by barium lactate in this procedure.

is required in the tube. The 40 ml. of the filtrate contains 4.8 ml. of 0.1 *N* HCl added during the carbon treatment.) To provide the 6 ml. of 0.1 *N* HCl required, add 1.2 ml. of the acid. Place the tube in a jacket of black paper, add from a buret or pipet 5 ml. of the FeCl<sub>3</sub> solution, make to the 50 ml. mark, and mix. (After the color has been developed, diluting for the purpose of reducing the color intensity is not permissible.) Fill a 4 inch cell, the walls of which are painted black, with the solution and read in the photometer, using filter No. 46.<sup>5</sup> Estimate the quantity of lactic acid present in the 40 ml. portion taken, from the standard curve of the instrument. If the quantity of lactic in the 40 ml. portion of filtrate exceeds the 12 mg. limit of the standard curve, repeat the estimation on a 10 ml. portion of remaining filtrate. The 10 ml. portion will contain 1.2 ml. of 0.1 *N* HCl and 4.8 ml. of the acid will have to be added to complete the 6 ml. required in the Nessler tube. Report lactic acid in terms of mg. per cent.

### VOLATILE FATTY ACIDS IN EGG PRODUCTS

#### I. APPARATUS<sup>6</sup>

A steam distillation assembly consisting of a boiler flask (3 liter) giving steam at a uniform rate so as to produce a constant rate of distillation, a distillation flask, a condenser, and 50 and 200 ml. volumetric flasks as receivers.

#### II. STANDARDIZATION OF THE DISTILLATION APPARATUS

So place the apparatus in the laboratory that it is free from drafts and sudden changes in temperature. Make a mark on the 3 liter boiler flask at the 1500 ml. level, fill to this mark with boiled distilled water,<sup>7</sup> heat to boiling, and boil for several minutes before starting a distillation. Transfer about 50 ml. of 0.1 *N* formic acid to the distillation flask, add 1 drop of H<sub>2</sub>SO<sub>4</sub> (1+1), and adjust the volume to 150 ml. with water. Connect condenser, insert steam inlet tube into distillation flask, and bring contents of this flask to incipient boiling by means of a burner. Next, connect steam inlet tube with steam supply of boiler and steam distil. So regulate the rate of evolution of the steam and height of small flame of burner under the distillation flask that the volume of the liquid in the distillation flask is kept constant at 150 ml. and the distillate is collected at the rate of 200 ml. per hour.<sup>8</sup> (A few trials with water in the distillation flask will show conditions necessary to maintain constant volume in distillation flask and rate of distillation.) Collect a 50 ml. portion of distillate, followed immediately by a 200 ml. portion. Transfer contents of the 50 ml. volumetric receiver to a 125 ml. Erlenmeyer flask, and those of the 200 ml. receiver to a 300 ml. flask, and titrate each to phenolphthalein end point with 0.1 *N* alkali. Make a blank determination, using 150 ml. of distilled water and 1 drop of H<sub>2</sub>SO<sub>4</sub> (1+1) in the distillation flask, collecting a 50 ml. and a 200 ml. portion and titrating. Correct the titrations of each distillate for the respective blanks. Calculate, in terms of percentage, the fractions of the total formic acid originally present, which were carried over into the 50 ml. and 200 ml. distillates.<sup>9</sup> Designate them as TF<sub>1</sub> and TF<sub>2</sub>, respectively.

<sup>5</sup> Use the same cell, photometer, and color filter as used in obtaining the standard curve. If a photometer is not available, comparisons can be made in the conventional manner with Nessler tubes (compare *This Journal*, 20, 135 (1937)).

<sup>6</sup> The apparatus illustrated in *This Journal*, 21, 685 (1938) is of practical dimensions and fulfils the requirements.

<sup>7</sup> Throughout the determination all distilled water used should be boiled.

<sup>8</sup> The period of collection may vary  $\pm 2$  min. The 150 ml. volume in distilling flask should remain constant within  $\pm 5$  ml. The boiling may be stopped to permit a test of the constancy of the 150 ml. volume by momentarily interrupting the steam supply.

<sup>9</sup> In *This Journal*, 25, 176 (1942), these were called "distillation rates." In these directions, the term distillation fractions is used.

Repeat the standardization, using 0.1 *N* acetic acid and also using 0.1 *N* butyric acid.<sup>10</sup> Calculate distillation fractions for these acids and designate those of the 50 ml. distillates as TA<sub>1</sub> and TB<sub>1</sub>, and of the 200 ml. distillates as TA<sub>2</sub> and TB<sub>2</sub> for acetic and butyric acids, respectively.<sup>11</sup> Calculate the ratio of TA<sub>2</sub>/TA<sub>1</sub> and designate as "C."

### III. PREPARATION OF BUFFER SOLUTIONS AND DETERMINATION OF DISTILLATION BLANKS

Prepare a buffer mixture of pH 8.6<sup>12</sup> by dissolving 12.404 grams of boric acid and 14.912 grams of KCl in water and diluting to 1 liter. (The salts should be as pure as possible.) 50 ml. of this mixture plus 12 ml. of 0.02 *M* NaOH (or its equivalent), made to 200 ml. with water, has a pH of 8.6. Transfer 60 ml. of this solution to a thoroughly clean 125 ml. Erlenmeyer flask, and 220 ml. of the same solution to a 300 ml. Erlenmeyer flask; add 3 and 5 drops of phenolphthalein, respectively, to the flasks and tightly stopper with rubber stoppers.

Determine the titration blank of a 50 ml. and a 200 ml. portion of distillate collected as directed in II from 150 ml. of distilled water and 1 drop of H<sub>2</sub>SO<sub>4</sub> (1+1) in the distilling flask. Transfer the 50 ml. distillate to a 125 ml. Erlenmeyer flask with about 10 ml. of water, and the 200 ml. distillate to a 300 ml. Erlenmeyer flask with about 20 ml. of water. Titrate the 50 ml. distillate with 0.01 *N* Ba(OH)<sub>2</sub> until the color matches that of the prepared 60 ml. buffer solution<sup>13</sup> and titrate the 200 ml. distillate to match the 220 ml. buffer solution. The indicator color should persist for about 10 seconds.

### IV. PREPARATION OF SAMPLE

*Liquid or Frozen Eggs.*—Weigh 80 grams into a tared 500 ml. Erlenmeyer flask, add about 150 ml. of water, and shake vigorously.

*Dried Eggs.*—Weigh 25 grams into a 250 ml. beaker and, with a heavy stirring rod, make into a smooth paste with water. Transfer the contents of the beaker to a tared 500 ml. Erlenmeyer flask, using a total of about 200 ml. of water.

To the samples add 25 ml. of normal H<sub>2</sub>SO<sub>4</sub> and shake for about 1 minute. Add 40 ml. of a 20% phosphotungstic acid solution, make to 350 grams with water, and shake for 1 minute. Filter through a 24 cm. folded filter paper. Weigh 150 grams of the filtrate into a 300 ml. Erlenmeyer flask, add an excess of Ag<sub>2</sub>SO<sub>4</sub> (approximately 1/2 gram), and heat to boiling on a hot plate under a water-cooled reflux condenser. Cool the flask with running water while still connected with the condenser. Transfer the contents of the flask to a 200 ml. volumetric flask with water, make to mark, shake, and filter through a folded filter paper. Test the filtrate for complete removal of chlorides with a few crystals of Ag<sub>2</sub>SO<sub>4</sub>. If all the chlorides have not been removed, add more Ag<sub>2</sub>SO<sub>4</sub> to the solution, shake for several minutes, and again filter, pouring back until bright.<sup>14</sup>

### V. DETERMINATION OF VOLATILE FATTY ACIDS

(a) *Distillation and titration.*—Pipet 150 ml. of the filtrate into the distillation flask of the apparatus, and if not already acid against congo paper make acid with

<sup>10</sup> Other volatile acids, such as propionic and isobutyric, can be included in a general standardization of the apparatus, but so far these other acids have not been encountered in either sound or decomposed eggs.

<sup>11</sup> Distillation fractions for standardization made in triplicate should check within a range of 1%.

<sup>12</sup> "Determination of Hydrogen Ions," by Clark, 2nd Ed., p. 107. Williams & Wilkins Co., Baltimore, Md.

<sup>13</sup> The alkali should be kept in heavily paraffined bottles. The titration outfit described in *This Journal* 21, 686 (1938), which can be readily constructed from material in the laboratory, has been found very convenient for this purpose.

<sup>14</sup> In rare cases when it is not possible to get a bright filtrate, the addition of filter-oel and refiltration will prove helpful.

H<sub>2</sub>SO<sub>4</sub> (1+1). Steam distil under the conditions specified under II, collecting one 50 ml. portion followed by one 200 ml. portion of distillate. Titrate each portion with 0.01 *N* Ba(OH)<sub>2</sub>, following strictly the procedure outlined under III.

(b) *Determination of formic acid.*—Combine the 50 ml. and 200 ml. portions of titrated distillate obtained above, add 2 drops of saturated Ba(OH)<sub>2</sub> solution, and evaporate to dryness on the steam bath. Add about 5 ml. of water to the residue and 1 ml. more of normal HCl than is necessary to liberate the volatile acids. Filter through a small paper into a 125 ml. Erlenmeyer flask with ground joint and wash the paper with water in such a manner that the total filtrate will equal 30–40 ml. Add 10 ml. of sodium acetate-sodium chloride mixture (25 grams of sodium acetate plus 12 grams of sodium chloride made to 500 ml.) and 10 ml. of a 5% H<sub>2</sub>Cl<sub>2</sub> solution. Connect the flask with a ground joint air condenser, place on the steam bath, and allow the liquid to react for 2.5 hours. Transfer the precipitate of calomel to a previously weighed Pregl filter tube<sup>15</sup> provided with a mat of asbestos about 2 mm. thick. Wash the precipitate with water, followed by alcohol, and dry for 0.5 hour at 100°C. Cool and weigh. The tube should be weighed with another Pregl tube as a tare, prepared with asbestos, and treated in the same manner as the one containing the precipitate.

The weight of calomel precipitated (mg.) from the combined distillates multiplied by .0975 gives the mg. of formic acid in the distillates. To calculate the total formic acid originally present in the aliquot of the sample in the distillation flask before distillation, divide this result by TF<sub>1</sub> plus TF<sub>2</sub>, as determined in II. Divide by 8.04 and multiply by 100 to obtain mg. per cent (mg./100 grams) of formic acid in the dried eggs being analyzed.

(c) *Computation of volatile acids other than formic.*—

(a) *Formic acid present.*—For the purpose of computing acids other than formic, the titrations of the total acid in the 50 and 200 ml. distillates, obtained under V(a), are corrected for the formic acid present. Convert the mg. of formic acid in the aliquot of the sample in the distillation flask before distillation (as calculated in V(b) to ml. of 0.01 *N* formic acid by dividing by 0.46. Multiply this result by TF<sub>1</sub> to obtain the ml. of 0.01 *N* alkali required to neutralize the formic acid in the 50 ml. distillate, subtract from the original titration, and designate the corrected titration as *t*<sub>1</sub>. Using TF<sub>2</sub> as the multiplier, correct the original titration of 200 ml. distillate in a like manner and designate as *t*<sub>2</sub>. Calculate the ratio *t*<sub>2</sub>/*t*<sub>1</sub> and compare with “C” as determined under II. If comparison shows a close agreement (±0.1), acetic and formic are the only acids present. The sum of the corrected titrations of the 50 ml. and 200 ml. distillates divided by the sum of TA<sub>1</sub> and TA<sub>2</sub> gives the ml. of 0.01 *N* acetic acid in the aliquot sample in the distillation flask before distillation. This divided by 8.04 and multiplied by 60 gives the mg. of acetic acid in 100 grams of the dried eggs being analyzed. If “C” is greater than *t*<sub>2</sub>/*t*<sub>1</sub> by more than 0.1, butyric acid is present.

(b) *Formic acid absent.*—Divide the titration of the 200 ml. distillate by that of the 50 ml. distillate determined as under V(a). If the result is in close agreement with “C” (±0.1), then acetic acid only is present, but if the result is less than “C” by more than 0.1, butyric acid is present. In the absence of butyric acid, compute mg. of acetic per 100 grams of dried eggs as directed above.

<sup>15</sup> The Pregl tube used is a glass tube approximately 75 mm long, the upper half of which is 7 mm. inside diameter and the lower end 3 mm diameter. A sintered glass disk about 3 mm. thick is fused into the constriction between the upper and lower halves. A convenient holder for the Pregl tube for filtration is a glass tube large enough to permit seating the Pregl tube at the constriction by aid of a rubber sleeve at the upper end of the holder. The holder passes through a rubber stopper into a suction flask. By means of a small rubber stopper the upper end of the Pregl tube is connected with a glass siphon through which the solution and precipitate are transferred to the filter. Wash water and alcohol from the flask are also passed through the siphon tube.

TABLE 3.—*Authentic liquid, frozen, and dried eggs*

SAMPLE NO.*	ORGANOLEPTIC			BACTERIOLOGICAL				CHEMICAL								SAMPLE NO.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
	ODOR		TASTE	VIABLE COUNT		MICROSCOPIC COUNT		ACIDS MG./100 GRAMS (DRY BASIS)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
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GROUP 1—GOOD EGGS																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
A1	None	None	Normal	0.26	0.56	0.0001	0.5	0.50	0.3	24	21	32	None	None	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace

\* The number of each sample is the same as that assigned in Tables 1 and 2.

† Results on liquid and frozen eggs are on basis of 1 ml., which is equivalent to 1 gram.

‡ "Off" is applied to a detectable odor in liquid or frozen eggs and to an abnormal taste in dried eggs not definitely assignable to decomposition.

When butyric acid is present along with acetic acid, the titration of the 50 ml. distillate (either corrected or uncorrected, depending upon whether formic acid is present) equals the sum of two products (1)  $TA_1$  times the total ml. 0.01 *N* acetic acid in the distillation flask at the start of distillation, plus (2)  $TB_1$  times the total ml. of 0.01 *N* butyric acid in the distillation flask at the start. Analogously, the titration of the 200 ml. distillate is the sum of  $TA_2$  times the total acetic plus  $TB_2$  times the total butyric in the flask. The values  $TA_1$ ,  $TA_2$ ,  $TB_1$ , and  $TB_2$  are constants for the particular apparatus as standardized under II, and the titrations are known. The quantities of acetic and butyric acids in the flask are unknown, and are to be calculated. These data can be set up as simultaneous equations and the unknown values found.<sup>16</sup> The calculated results will be in terms of ml. 0.01 *N* acid. The ml. 0.01 *N* acetic acid divided by 8.04 times 60 equals mg. acetic acid per 100 grams of the dried eggs being analyzed. The ml. 0.01 *N* butyric divided by 8.04 times 88 equals mg. butyric acid per 100 grams of dried eggs.

### DISCUSSION OF RESULTS

The bacteriological, chemical, and organoleptic data obtained in this investigation on liquid, frozen and dried eggs and which are regarded as useful in objective judgment of decomposition are presented in Table 3. In the bacteriological studies the members of the coliform group are not reported, as this group, while widely used as an index of sanitation, has but limited value as an index of decomposition. As another investigator has reported (21), it was found that this group is largely destroyed during the drying process. With frozen eggs it has also been reported that the coliform index and the total plate count are roughly parallel (22).

The viable bacterial counts in liquid eggs and in the frozen eggs made therefrom are generally of the same magnitude (Table 3). Although some variations occur with freezing, the count in general varies directly with the quality of the product. With dried eggs, too, there is a general tendency for the plate count to increase as decomposition develops, but the increase is too variable to be a reliable index of the original condition of the liquid eggs. This conclusion is supported by the investigator, referred to above (21), who states further that the viable count decreases on storage, particularly with samples of high bacterial content. This latter finding is also reported by others (23).

The microscopic bacterial count, which includes both living and dead cells, appears to be just as reliable an index of decomposition of liquid and frozen eggs as is the viable plate count. On dried eggs it is a much more reliable index of this condition. During the drying of the eggs there appears to be an appreciable decrease in the microscopic count. Whether this is due to destruction of bacterial cells or to a loss of their ability to stain has not been determined. The decrease, however, is too variable to permit an accurate estimate of the bacterial content of the liquid eggs from the count on the dried powder. An important advantage of the microscopic

<sup>16</sup> *This Journal*, 25, 180 (1942).



count is that there is no appreciable change on storage over a period of two years at room and at refrigeration temperature (40°F.). Others have also shown this to be true over a period of ten months at temperatures ranging from 0° to 110°F. (23). In no instance did dried eggs show a microscopic count exceeding 10 million per gram or frozen eggs 5 million per gram when they were prepared from sound, raw material. In all cases where these counts were exceeded decomposed or rotten eggs had been incorporated in the product or the eggs had been subjected to conditions after breaking-out which permitted them to sour.

The chemical results for acidity of ether extract, volatile-base nitrogen, decomposition quotient, sugars, pH and total solids are not included in Table 3. In these experiments, the acidity of ether extract was found to have no significance. There was a tendency for the pH to be below 7 with eggs which had soured on standing, but the differences were not sufficiently consistent to be of value in demonstrating decomposition. Further, others have shown that storage for six months may change dried eggs by as much as 1.0 pH (24). The decomposition quotient in no instance approached the values proposed by its authors as indicating decomposition. This may be because the present experiments did not involve the cheesy type of decomposition referred to in the original study. Significant results on sugar content were found on Samples B5, RR2, and RR3, where none remained in the liquid egg after the holding period. With the two other samples in this group (RO2 and RO3) the sugar content was well below the average. It is significant that the samples in which the sugar was completely destroyed are the ones highest in bacteria and also in developed acids. Likewise experience with many commercial samples show that those of high count and acid content contain no sugars. The natural sugar of the egg is destroyed as bacterial growth proceeds. These findings show the need for further studies on the changes in the constituents of the egg caused by the destructive action of microorganisms associated with the various types of egg decomposition, and on the identification and the determination of the end products formed. The volatile-base nitrogen was found to increase significantly in sour liquid eggs, and to a more limited extent in some samples containing added rotten eggs. However, drying brought the results of all samples within so narrow a range that certain detection of decomposition by this method was not possible. The diagnostic value of volatile-base nitrogen in frozen eggs should be evaluated by further study. Total solids were determined to permit calculations of the results to a dry basis so that direct comparison could be made between the batches in their liquid and dried states.

The data presented show that edible eggs contain no formic, not more than a trace of acetic, and only limited amounts of lactic acid. There is no material increase in these acids when sound liquid eggs are held for a day under refrigeration or for short periods at high temperature, or when

they are frozen and thawed before drying. Further, eggs so treated showed no odor of decomposition. Fish meal and oil, and linseed or cottonseed meals in the diet of hens do not affect the odor or taste of eggs, nor their content of these acids. There is no evidence that lactic acid content is materially affected on drying or that more than a mere trace of formic acid is formed. However, acetic acid is formed in appreciable amounts during drying. Over-heating during drying does not further increase these acids.

As is to be expected, the chemical results on the frozen-egg samples agree with those on the liquid samples. When liquid eggs become sour on standing at a temperature above that of refrigeration, formic and acetic acids are formed and the normal lactic acid content is substantially increased. This development of acids is accompanied by rapid bacterial increase. The odor of the liquid eggs that were allowed to become sour was repulsive and acid-like, resembling the smell of garbage in an active state of spoilage, and the taste of the dried eggs made therefrom was strong and vinegar-like, repulsive almost to the point of nausea.

The agreement of bacteriological and chemical findings with the organoleptic evidences of incibility on dried eggs has been uniformly excellent in hundreds of commercial samples that have been routinely examined. Any sour taste has been accompanied by high bacteria and acid contents, the quantities increasing with increased sourness.

The determination of volatile and lactic acids in either liquid or dried eggs appears to be of little value for demonstrating the presence of decomposed or objectionable shell eggs added in limited amounts. This is doubtless because decompositions in shell eggs are of so many different types. That these acids are not always formed is supported by tests made on good and obviously inedible individual shell eggs. No formic or acetic acid was found in 18 individual good eggs, and the lactic acid range was only from 3 to 7 mg. per 100 grams of liquid egg. Results on 14 inedible eggs are given in Table 4. With the quantities of acids that may be present in an inedible egg as low as these here found, it is obvious that a very considerable percentage of bad eggs could be present in a batch without detection because of their dilution by the sound eggs. With three batches containing added inedible eggs, namely RM, RB, RU, the odor of the liquid established their inedibility although results on acids did not. Two of these samples tested in the frozen state confirmed the value of the "drill and smell" test to detect decomposed eggs in the frozen product. The smell test has been demonstrated in other unpublished studies to be reliable and valuable for detecting inedible shell eggs in both liquid and frozen eggs. The absence of chemical evidence obviously does not detract from the soundness of interpretations based on smell. Methods to detect and determine *all* possible end products of decomposition are not available.

TABLE 4.—*Acids in shell eggs*

NO.	DESCRIPTION	FORMIC	LACTIC	ACETIC
		mg./100 gram*	mg./100 gram*	mg./100 gram*
1	Odorless addled eggs	None	143	17
2	Odorless addled eggs	None	7	None
3	Odorless addled eggs	None	6	None
4	Odorless addled eggs	2	6	11
5	Odorless addled eggs	None	6	None
6	Odorless addled eggs	None	6	None
7	Mixed rots	None	74	42
8	Mixed rots	2	6	4
9	Mixed rots	3	15	8
10	Mixed rots	8	6	13
11	Mixed rots	15	10	22
12	Mixed rots	13	11	20
13	Mixed rots	9	6	19
14	White rots	1	6	3

\* Liquid egg basis.

These chemical studies demonstrate that dried whole eggs prepared from sound, wholesome, good, shell stock, properly broken-out and handled under sanitary condition, with proper refrigeration, should contain no formic, not more than 50 mg. of lactic, and not more than 65 mg. of acetic acid per 100 grams on a dry basis.

#### COMMERCIAL SAMPLES

The application to commercial samples of the bacteriological and chemical determination shown to be of value on authentic decomposed dried eggs was studied through visits to a number of dried-egg establishments in active production. Some plants were using current-receipt eggs, others storage eggs, and still others combinations of the two. Occasionally frozen eggs were used to supplement the supply of shell-stock. The eggs were observed at each stage of the preparation, from the candling, breaking, mixing, and churning on into the pressure pump and dryer. Temperatures and odors of the liquid eggs in the mixing or holding tanks were recorded from time to time during the visit. Samples were taken of the liquid egg going to the dryer, and a microscopic count was made. When it was certain that the liquid egg, the preparation of which had been observed, was being dried, samples of the dried eggs were taken to be analyzed later. Table 5 presents the data on such samples. None of the samples showed a decomposed flavor by the taste test. In only two cases were significantly high bacteria counts encountered, namely Samples A and G. These samples also exceeded the maximum of 50 mg. of lactic acid per 100 grams established by the experimental authentic packs. Sample A was from a firm employing a "wet collector," in which the

TABLE 5.—*Commercial samples—authentic*

SAMPLE	ACIDS MG./100 GRAMS (DRY BASIS)			MICROSCOPIC COUNT (MILLIONS)	
	LACTIC	FORMIC	ACETIC	PER GRAM DRIED EGG	PER ML. LIQUID EGG
A	150	None	23	202.0	2.5
B	47	None	19	17.0	8.0
C	44	None	16	28.0	42.5
D	30	None	19	63.0	190.0
E	38	None	17	1.5	6.0
F	49	None	16	0.5	1.0
G	63	None	23	110.0	410.0
H	38	None	16	31.0	40.0
I	47	None	20	1.5	3.0
J	42	None	19	5.5	8.0
K	47	None	17	0.5	1.5
L	47	None	19	1.0	1.0
M	23	None	30	1.5	4.5
N	37	None	24	1.5	2.0
O	24	Trace	35	0.5	1.5
P	44	None	32	0.5	1.0
Q	40	None	32	1.5	4.5
R	37	None	29	0.5	2.5
S	37	None	29	0.5	2.0
T	24	None	24	4.5	5.0
U	36	Trace	27	4.0	8.0
V	26	None	20	3.0	5.0
W	23	None	23	0.5	0.5

fine powder carried out with the exhaust air was exposed to the incoming liquid egg and returned to the dryer. The output of this manufacturer consistently showed excessive bacterial counts, and shortly after the visit when Sample A was collected this equipment was replaced by one of the conventional types used extensively in the industry. This change materially reduced the bacterial content of the output and brought it into agreement with that of other manufacturers. With Sample G the eggs were broken-out in a plant 150 miles distant from the drying plant. During the trip of several hours, the only provision for refrigeration was a few blocks of ice on some of the metal barrels. The temperature of the liquid on arrival at the drying plant was from 50° to 60°F. The odor of the liquid was indicative of incipient decomposition. The circumstances surrounding these two samples account for the abnormal bacterial and chemical results. Samples B, C, D, and H gave microscopic counts ranging from 17 to 63 million but they were in the normal range for acids. The counts exceed the 10,000,000 maximum found in the experimental authentic packs, but no condition of the eggs or the method of handling them was observed which could account for the increases. An extremely liberal limit

of 100,000,000 bacteria per gram is therefore suggested as a maximum for edible commercial dried egg. These experiments with commercial dried eggs show that the use of the suggested maximum microscopic count and of the maxima previously suggested for volatile and lactic acids, will not condemn any product made from sound, edible eggs.

### SUMMARY

Bacteriological and chemical criteria for some forms of decomposition in liquid, frozen and dried eggs have been developed.

With liquid and frozen eggs a microscopic count of over 5,000,000 per gram, with determinable amounts of either formic or acetic acid or lactic acid in excess of 7 mg. per 100 grams of liquid egg, demonstrates the presence of decomposed eggs.

With dried eggs a microscopic count of over 100,000,000 per gram, with determinable amounts of formic acid and acetic acid over 65 mg. and lactic acid over 50 mg. per 100 grams (on the dry basis) demonstrates the presence of decomposed eggs.

Whenever the taste of dried egg was sour, the bacteriological and chemical results have been well above these maxima.

Certain types of decomposed eggs can be present in liquid, frozen, or dried eggs without being detected by these bacteriological and chemical methods.

The smell test is reliable for establishing a decomposed condition in liquid and frozen eggs in the absence of other criteria.

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## NOTES

### Tests for Completeness of Extraction of Tobacco Alkaloids from Plant Materials\*

In the extraction of alkaloids from plant materials it is often imperative that a reliable qualitative test be used to prove that the extraction is complete. The use of Prollius' fluid<sup>1</sup> is not convenient, as it requires too long a time before the results are known. Since the solvent may give incomplete extraction or the plant material may not be sufficiently divided to permit complete extraction, some other procedure should be used in preference to further extraction and testing of the extract solvent.<sup>2</sup> The following slight modification of the method recommended by Markwood and Barthel<sup>3</sup> for the complete disintegration of tobacco samples before extraction with benzene may be used in the case of plant materials containing the tobacco alkaloids. Approximately 5 grams of the marc is treated with 10 ml. of strong (9+1) sulfuric acid, whereby the cellular structure is broken down with the formation of the sulfates of any remaining organic bases, and then mixed for several minutes; 25 ml. of water is added, and the undissolved portion is removed by filtration. The filtrate is neutralized with NaOH (300 g./liter), just acidified with 10% HCl, and tested with silicotungstic acid solution. If salts should separate during the neutralization, the clear supernatant liquid must be decanted before the reagent is added. The test solution and the reagent are warmed and then cooled with strong agitation. No precipitation indicates less than 0.01 per cent of nicotine, nornicotine, or anabasine left in the marc.

Protein materials in the marc do not appear to interfere with the test. Other alkaloid reagents may be substituted for the silicotungstic acid if desired. The test is simple, rapid, and adaptable to the reagents at hand.

\* By C. V. BOWEN and W. F. BARTHEL (U. S. Department of Agriculture, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine, Beltsville, Maryland)

<sup>1</sup> "Allen's Commercial Organic Analysis," 5th ed., Vol. VII, p. 6 P. Blakiston's Son & Co., Philadelphia (1929).

<sup>2</sup> *Ibid.*, p. 10.

<sup>3</sup> Markwood and Barthel, *This Journal*, 26, 280-283 (1943).

### Preparation of Small Animals for Analysis\*

In studies of the utilization of the energy of feeds for growth by chickens and rats,<sup>1</sup> it was necessary to analyze large numbers of small animals, chiefly for protein and fat.

Several different methods of preparing the animals for the analyses to be made were tried. Some chickens were dried easily in a vacuum oven and reduced to a fine powder in a Wiley mill, but there was danger of loss of fat in grinding, and the samples took up sufficient water to change the moisture content. Rats were dried in desiccators over sulfuric acid, partly extracted with ether, dried, and ground again. This procedure was very slow, and some of the samples had a bad odor before the drying was completed. Considerable calculation was required to convert the analyses to the original basis.

The fresh chickens or rats, after the intestinal contents had been removed, were ground in a meat chopper for direct analysis. In a comparatively short time water separated from the sample, making it impossible to weigh out representative portions. After trying several absorbents, the writer found that 2.5-3 per cent of filter paper absorbed the water and maintained a suitable consistency.

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\* By G. S. FRAPS (Texas Agricultural Experiment Station, College Station, Texas)

<sup>1</sup> Texas Agr. Expt. Sta. Bulls. 571, 600, 625, 632.

Analyses were begun the day the samples were ready, but it was necessary to preserve them as long as additional check analyses were required. Boric acid was used for this purpose, but it was found to be not entirely satisfactory.

Since January 1940,<sup>2</sup> the animals have been cooked in an autoclave. The method used is similar to that employed by Gladys Leavell,<sup>3</sup> but with the important differences that filter paper was added to absorb water and correction was made for loss of water in autoclaving. This method has been used most generally for chickens or rats weighing less than 500 grams, but it has also been applied to a few chickens, 12-18

TABLE 1.—*Live weight, empty weight, weight of filter paper, and loss in cooking chickens in preparation for analyses*

	LIVE WEIGHT	EMPTY WEIGHT	FILTER PAPER ADDED	LOSS IN COOKING
<i>Sample No.</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
64465	176.8	168.6	5.0	9.7
64468	198.5	193.1	5.0	9.8
64471	240.2	232.2	6.3	13.1
64474	182.6	174.6	5.0	10.7
64477	170.0	162.7	5.0	7.7
64480	175.7	170.3	5.0	10.1
64317	147.9	141.0	3.7	3.2
64325	278.4	268.2	6.3	6.0
64332	246.0	229.0	6.3	7.6

TABLE 2.—*Calculation to empty weight of chickens*

	<i>grams</i>	
Weight after cooking	163.9 (A)	
Filter paper added	5.0 (B)	
Total	168.9 (C)	
Empty weight	174.6 (D)	
Correction factor, C divided by D		0.9674 (F)
Corrected protein (protein × F)		21.93%
Corrected fat (Fat × F)		2.54%

weeks old, weighing as much as 1445 grams. These larger animals were divided and autoclaved in two half-gallon jars. Samples weighing 3.5 grams were used for the protein determinations, and others weighing 4.0 grams were used for the fat determinations.

Table 1 contains data secured with this method, and Table 2 illustrates the method of calculation of the data to the original empty weight.

#### METHOD

The procedure as finally used is as follows:

##### PREPARATION OF FILTER PAPER

Moisten 200 grams of filter paper with 170 ml. of HCl (6 ml. to 250 ml. of water), and dry at 50°C. Grind in a Wiley mill, using a 1 mm. sieve.

<sup>2</sup> Texas Agr. Expt. Sta. Bull. 625.

<sup>3</sup> *This Journal*, 25, 159 (1942).



## PREPARATION OF CHICKENS OR RATS

Record live weight of animal. Kill the animal with  $\text{CHCl}_3$ , remove the digestive tract, and cut open stomach or gizzard and remove any food. Straighten out the digestive tract and remove contents by pressing lightly with a spatula on a flat surface and pulling gently through the opening. (In this way all the contents can be removed without splitting the digestive tract.) Be careful not to press the spatula down so hard as to squeeze out liquids. Weigh again and put into a fruit jar of suitable size and having a numbered aluminum tag. Weigh jar and contents. Tighten the cover, place in autoclave, raise pressure to 15 pounds, and maintain this pressure for 3 hours. Turn off the heat and allow the pressure to come to atmospheric pressure. Remove jar from autoclave, allow to cool overnight, and weigh. (The decrease in weight gives the loss of water.) Grind the animal through a meat grinder, and add 2.5% of filter paper during the grinding so as to distribute it evenly. (It is convenient to weigh the filter paper into beakers, using 2.5 grams per animal with a live weight less than 100 grams, 3.8 grams for those animals weighing 100–150 grams, and so on.) Grind the sample as rapidly as possible to avoid excessive evaporation of water and continue to grind as many times as necessary to produce a good sample. Mix the ground sample with a spatula, place in fruit jars tightly closed, and keep in the refrigerator except when a part is being weighed for analysis. (It is well to weigh the sample just after it is ground, to ascertain if there is a normal loss in preparation. There will be some loss owing to the sticking of the material to the meat grinder and other utensils, and also some evaporation of water.)

## Photographic Action of Radium and Other Substances\*†

A paper by George L. Keenan<sup>1</sup> gives a complete review covering the period from 1842 to 1925 of investigations dealing with the effect certain non-radioactive substances have on the photographic plate. Some of these substances are paper, marble, feathers, cotton, zinc, magnesium, iron, anthracene, and anthraquinone. It was found that each of these substances had at least one of the following properties: self-luminosity, radiance from previously absorbed light, emission of metallic vapors, or chemical activity.

In 1925 Baughman and Jamieson<sup>2</sup> observed that fatty acids did not react on photographic plates, but that when these same fatty acids were exposed to the sunlight for 5–6 hours and then to a photographic plate they had developed the property of producing a reaction with the sensitized coating of the plate. The consensus of opinion of these investigators was that the photographic plate is affected by a vapor that arises from the substance and reacts with the sensitized film of the plate.

The writer has compared the effects on photographic films of a dilute radium solution and certain non-radioactive substances. The samples used were linseed oil, hydrogen peroxide, and kerosene. The linseed oil, protected from daylight, was poured into an evaporating dish, which was then placed in a desiccator and kept in a dark room. A film (sensitized side to the oil) was placed over the top of the dish, and the dish was allowed to stand in the dark for about 16 hours, after which the film was developed and printed.

The next day the same sample of linseed oil, protected from daylight, was transferred to a Petrie dish, which was then placed in a desiccator kept in a dark room. The sensitized side of a film facing a black paper cut-out was placed over the dish in the desiccator. This film was also exposed for 16 hours, after which it was developed and printed.

\* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 27, 28, 1943.

† By ANNA E. MIX, Food and Drug Administration, Federal Security Agency, Washington, D. C.

<sup>1</sup> *Chem. Rev.*, 3, 95 (1925).

<sup>2</sup> *J. Oil & Fat Ind.*, 2, 25–28 (1925).

Kerosene and peroxide were used in the same manner, except that a second film was placed under each dish used. While the film on the top of each dish was affected, the film under the dish was not affected. At the same time a film was placed on top of a lead box having a cut-out design and containing a glass tube of one microgram of radium solution. This film showed a very definite reproduction of the design. The same lead box, containing the same radium sample, was inverted and placed on a glass plate covering a sensitized film. This also showed a definite reproduction of the design. The radium solution showed a very definite reaction through the glass plate while the linseed oil, kerosene, and peroxide gave no reaction.

The photographs made from these negatives show that a definite reaction took place on the sensitized film when these substances were directly exposed to it, but that the films were not affected when a glass plate was interposed between the substance and the film except when the radium solution was used. The radium not only reacts with the film as in the case of the other substances, but it also reacts when the radioactive source is covered with a glass.

The non-radioactive substances that acted on the film were then placed in 2 ounce prescription bottles and exposed separately and collectively to a Geiger-Müller quantum counter. No reaction was recorded by this instrument, which is sensitive to one microgram of radium at one meter distance from the counter tube.

### A Rapid Method for Determination of Fat Content of Meat\*

In meat dehydration processes it is important to control the fat content of the finished product. The conventional methods for the determination of fat require several hours, and they are not suitable for control work in commercial establishments. For such work a method must be rapid and yet sufficiently accurate to keep the product within specified limits; it should also be suitable for use with raw and precooked meat as well as the dried or partly dried product. The following method is submitted for this purpose.

#### METHOD

##### APPARATUS

The apparatus consists of a medium or fine porosity, sintered-glass filter plate, fused into a separatory funnel of about 250 ml. capacity to permit repeated extractions without transfer of sample (Figure 1).

##### DETERMINATION

A sample of finely ground meat, weighing 5 or 6 grams, is placed in the funnel, and 50 ml. of anhydrous ether is added. The funnel is shaken for about 60 seconds, and the ether is drawn off into a weighed flask of 250 or 300 ml. capacity, fitted with a 24/40 standard taper joint.<sup>1</sup> The ex-

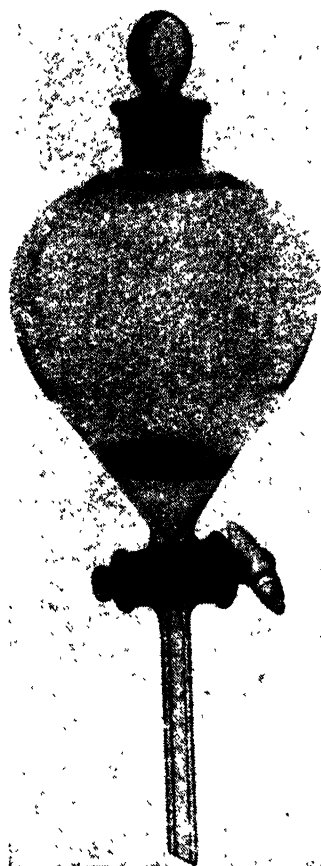


FIG. 1.

\* By A. J. EANSR (Agricultural Research Administration, United States Department of Agriculture, Washington, D. C.)

<sup>1</sup> Equipment of Scientific Glass Apparatus Co., Bloomfield, N. J., was found suitable, condenser J 1220 being used.

TABLE 1.—*Comparison of A.O.A.C. method and proposed rapid method*

SAMPLE	A.O.A.C. METHOD	RAPID METHOD	DIFFERENCE
	<i>per cent</i>	<i>per cent</i>	
<i>Raw meats</i>			
1635	14.9	14.5	-0.4
1656	16.0	16.9	+0.9
1659	8.9	9.4	+0.5
1672	7.5	6.9	-0.6
1673	18.7	19.0	+0.3
1690	8.7	8.1	-0.6
1636	10.0	9.1	-0.9
1574	18.8	18.4	-0.4
1589	17.0	16.3	-0.7
<i>Precooked meats</i>			
1691	11.4	11.2	-0.2
1692	12.8	12.9	+0.1
1694	14.1	14.2	+0.1
1695	13.8	14.0	+0.2
<i>Dehydrated meats</i>			
1634	22.4	23.4	+1.0
1586	31.1	31.2	+0.1
1633	38.5	40.2	+1.7
1666	33.8	34.6	+0.8
1681	22.9	23.9	+1.0
1696	22.5	22.4	-0.1
1697	39.4	39.8	+0.4
1710	37.7	37.8	+0.1
1730	12.1	13.2	+1.1
1739	26.1	27.1	+1.0

traction is repeated once with 50 ml. of ether and three times with 30 ml. The flask is then connected to a condenser<sup>1</sup> for the recovery of the ether, which is rapidly boiled off. The flask that contains the ether extract is placed in a vacuum oven, which is kept at 100°C. and under a vacuum of 25 inches for 30-45 minutes. The flask is then cooled and weighed. From the difference in weight the percentage of fat is calculated. The time required for determination of fat by this procedure is 1.5-2 hours.

Raw and precooked meat samples filter readily, and if some dehydrated samples are difficult to filter sand can be mixed with the meat sample and suction applied to increase filtration rate. After the extracted sample is removed from the funnel the filter plate is cleaned by reverse flushing. This procedure effectively removes small particles from the sintered-glass filter.

In Table 1 a comparison is shown between results obtained by the A.O.A.C. method and those obtained by the method presented here. The agreement is very good in most cases. These results demonstrate the adequacy of this rapid method in control work for determining the approximate fat content of raw, precooked, and dehydrated meats.

## BOOK REVIEWS

**Glue and Gelatine.** By PAUL I. SMITH. Chemical Publishing Company, Inc., Brooklyn, New York (1943). 145 pages. Price \$3.75.

The book, as the author states is "... written more as a primer for the practical man than an enlightened and original book for the research chemist. . . ." The topics covered are: Early History, Histology of Skin and Structure of Proteins, Chemistry of Proteins, Raw Material for Glue and Gelatine, Preliminary Processes, Extraction of Gelatine, Filtration, Clarification and Addition of Chemicals, Evaporation, Recovery and Purification of Bone Fat and Skin Greases, Properties and Qualities of Glue and Gelatine, Plastics for Gelatine Plant Construction, Application of Glue, Gelatine for Photographic Uses, Gelatine in the Food Industry, and Miscellaneous Uses. The book will be useful to persons desiring a brief general description of the subject.—H. R. KRAYBILL.

**The Dispensatory of the United States of America.** The 23rd Edition by HORATIO C. WOOD, Jr., M.D. and ARTHUR OSOL, Ph.D., assisted by HEBER W. YOUNGKEN, Ph.D., and LOUIS GERSHENFELD, D.Sc. J. B. Lippincott Company, Philadelphia 5, Pennsylvania. 1943. 1881 pages. Price \$15.00.

Although this work in various editions has been in existence for more than 110 years, and the publication is commonly called "The United States Dispensatory," it should be understood that the work is privately owned and privately published, and that the Government of the United States of America has no part whatever in its authorship, ownership, or publication.

This edition is divided into three parts. Part I includes the drugs described in the Pharmacopoeia of the United States, The National Formulary, and the British Pharmacopoeia; Part II describes drugs not included in the works mentioned, but it also includes a section on diagnostic tests; Part III is devoted to processes, reagents, solutions, and tables of the U. S. Pharmacopoeia or the National Formulary. The information is intended to be encyclopedic. It includes titles, definitions, synonyms, sources of drugs, manufacturing processes, history, chemical constituents, tests for identity and purity, and assay methods where practicable. The information on dosage, toxicology, incompatibilities, and therapeutic uses is of particular value to pharmacists and nurses. The sections devoted to the history, preparations, and marketing of drugs are well written and should be of value to any one interested in drugs, if only in a general way. Many drugs that have gone out of use or nearly so are described for the historical value of this information.

Since the 1937 edition of the Dispensatory appeared, the therapeutic properties of a new class of drugs have been discovered. These are so-called "sulfa" drugs, and a section is devoted to a review of their chemistry and therapeutic properties. Other new drugs discussed for the first time are diethylstilbestrol, diodoquin, demerol, amylcaine, pentothal sodium, pontocaine, nicotinic acid, nicotinamid, and penicillin.

The section on vitamins has been revised. Attention is given to the antimalarials, quinacrine hydrochloride, and pamaquine naphthoate, and their relative values in the treatment of malaria as compared with quinine are discussed.

Since most states now require every practising pharmacist to have a copy of the Pharmacopoeia and the National Formulary in his prescription department, a considerable saving in paper and printing costs of the Dispensatory might be made by omitting the descriptions of the official drugs in Part I. A statement that the drug is official would be sufficient for the pharmacist, and the book would be just as useful as it is now. Likewise, most of the tables in Part III could be eliminated, since they are copied chiefly from the Pharmacopoeia without useful comment.

The section on diagnostic tests is clear, concise, and informative.

In some respects, the most interesting part of the Dispensatory is Part II—drugs and preparations not found in the U.S.P., N.F., or B.P. This is "The Old Curiosity Shop" of the book. Here are the old and the new in medicines; the obsolete and the latest fads—with all grades between; the hosts of synthetics invented, tried and mostly found wanting; a great variety of plant extractives used as charms, cosmetics, medicines, or arrow poisons by the natives of tropical countries; and numerous substances, not drugs, which are or may be hygienic or commercial menaces.

The chapter on Clinical Laboratory Reagents in Part III comprises 33 pages. It describes the various tests commonly employed in clinical technology in sufficient detail for use by the nurse or technician without recourse to other manuals. A new and praiseworthy feature is that the running heads and page numbers are printed in bold-face type. This sharply differentiates them from the text and should prove helpful in finding subjects. An index of over 30,000 entries gives some concept of the range and comprehensiveness of the subject matter in this Dispensatory.

The Dispensatory will continue to be a guide and reference work for the pharmacist, the nurse, the clinical laboratory technician, and the student of medical and pharmaceutical history. It will be useful to the busy physician who wishes to refresh his memory on the newer remedies. Presumably, it will not be very helpful to the drug analyst who is on the lookout for the latest critical methods.—L. E. WARREN.





DR. SAMUEL PALKIN, 1890-1943

## SAMUEL PALKIN

On the afternoon of May 2, 1943, there was ended in his 54th year, the life of Samuel Palkin. Death, from a heart attack, came suddenly and unexpectedly and was a profound shock to relatives and to many friends in the Department of Agriculture. Dr. Palkin had apparently completely recovered his normal health after a siege of virus pneumonia only shortly before, so that the suddenness of his demise added to the surprise and shock.

Dr. Palkin was born in Kovno, Russia, on February 22, 1890, and was brought to the United States by his parents in 1891. The family settled in Stamford, Connecticut, where his first chemical training was obtained under the tutelage of the late Frederick H. Getman in the local high school. Dr. Palkin would recall with obvious pleasure experiences in Dr. Getman's laboratory, and it was plain who had supplied the inspiration which led him to choose a career in chemistry. Dr. Palkin obtained his Ph. B. in Chemistry at Yale in 1910 and spent the next year as a chemist in the employ of Tiffany and Company. In 1911 he was appointed assistant chemist in the Drug Division of the Bureau of Chemistry of the United States Department of Agriculture in Washington, D. C., and the remaining 32 years of his life were spent in Washington in the employ of this Department. There he took advantage of the opportunity of attending night graduate classes at the George Washington University and was awarded the M. S. degree from that institution in 1913 and the Ph. D. degree in 1915. The award of this degree was promptly followed by his marriage on June 22, 1915, to Miss Rose Epstein, also of Stamford, Connecticut, and Washington, D. C.

From 1911 to 1918 Dr. Palkin served with the Drug Division of the Bureau of Chemistry. He then joined the old Color Laboratory of the Bureau of Chemistry as an organic and physical chemist, but returned to the Food, Drug and Insecticides Division in 1922. Here he remained until 1929. His outstanding researches in the Drug Control and Color Investigation Laboratories led to his appointment to the Naval Stores Research Division of the Bureau of Chemistry and Soils in 1929. Here he remained, and in February, 1943, only three months before his death, he was appointed Chief of the Naval Stores Research Division. As chief he not only attended to the administrative duties and actively directed the research work of the Division, but he also maintained a laboratory bench at which he would work whenever he could seize time from more pressing duties to play with his own ideas before turning them over to his associates for further development. His important investigations upon the chemical constituents of turpentine and rosin and upon methods for analyzing and improving the manufacture of naval stores have vastly widened these fields of science and technology.

Dr. Palkin was active to the very last. It was characteristic of him that only the day before his death, on Saturday May 1, he completed his annual report on the work of the Division to the Chief of the Bureau, leaving his affairs entirely in order for his successor. He was author or co-author of more than 60 papers, most of them published in the *Journals of the American Chemical Society*. Seven patents had been issued to Dr. Palkin as inventor or co-inventor, and more than a dozen were pending at the time of his death—all of them assigned to the Department of Agriculture for the free use of the people of the United States.

An adequate review of the diversified subjects that have been investigated and reported upon by Dr. Palkin is beyond the limits of this obituary. His chief contributions were in four fields, viz. metals, drugs and medicinal products, photosensitizing dyes, and naval stores products. Some idea of the scope of his work may be obtained from the following excerpts of a few titles of his journal articles and patents:



"On the Stability of Nitrous Ether"; "The Synthesis of Dicyamine A"; "The Separation of Lithium from Other Alkali Metals"; "Azo Dyes from Alkaloids of Ipecac Root and Their Identification by Means of the Spectroscope"; "Crystallizable Chavicol from the Oil of Bay"; "The Resin Acids of American Turpentine Gum"; "On the Nature of Pyroabietic Acid"; "Vapor Phase Thermal Isomerization of  $\alpha$ - and  $\beta$ -Pinenes"; "An Efficient Column Suitable for Vacuum Fractionation"; and "A Precision Oil Gauge."

Following his connection with the Bureau of Chemistry in 1911, Dr. Palkin was an almost constant attendant at the annual meetings of the Association of Official Agricultural Chemists. His first work for the Association was that of collaborator in the report on morphine<sup>1</sup> at the 1912 meeting. In the following year W. O. Emery and Palkin presented their paper on "Estimation and Separation of Antipyrin from Various Synthetic Products by Means of its Periodide."<sup>2</sup> This was the beginning of a valuable series of "Studies in Synthetic Drug Analysis" by the same authors, of which may be cited "Estimation of Antipyrin,"<sup>4</sup> "Estimation of Caffeine and Antipyrin in Admixture,"<sup>4</sup> and "Periodides of Antipyrine, Iodoantipyrine, and Pyramidone."<sup>6</sup> At the 38th and 39th annual meetings of the Association Palkin as an Associate Referee on Drugs presented papers on "Methods for the Determination of Phenolphthalein"<sup>6</sup> and "Phenolphthalein and Chocolate Preparations."<sup>7</sup> At the 41st meeting of the Association Palkin, still serving as Associate Referee, presented his "Report on Santonin,"<sup>8</sup> and at the 42nd meeting H. R. Watkins and Palkin in continuation of their studies on alkaloids read a paper on "Errors in Analysis of Alkaloids Caused by Presence of Fatty Acid and Soap."<sup>9</sup> This was the last of Palkin's numerous contributions to the work of the A.O.A.C. on drug analysis, as shortly thereafter he abandoned this field for later researches on dye stuffs and naval stores. The last report in which he participated for the Association was a joint contribution by M. B. Matlack and himself at the 50th meeting on "Total Neutral and Unsaponifiable Matter in Rosin with Data Relative to Mechanical Methods for their Determination."<sup>10</sup>

Dr. Palkin contributed importantly to knowledge of the resin acids present in American turpentine gum and of the so-called pyroabietic acid. He pioneered in the development of chemical methods for the stimulation of gum flow from trees to increase the yield of pine gum. He was a zealous worker and a generous colleague—always ready to carry his share of the work and let the credit go. Especially with younger men, he was insistent that they be given a full share of the credit in all publications, even if it meant leaving off his own name. Among the outstanding characteristics of the man were his faithful devotion and loyalty to his family, his friends, and his work.

Dr. Palkin's quiet exterior hid a keen sense of humor—the dry kind that sought out and poked fun at fallacies in people's pretensions and showed the funny side of each day's experiences. Often a tense moment in a discussion or conference would be broken by a sly joke, spoken with a perfectly blank countenance. The spontaneous laugh that would follow the moment of surprise at the apparent irrelevance would ease the tension, and the discussion would then frequently be concluded in short order. His seeming composure served to cover up his deep feeling for humanity. Many of us hear of another's sufferings or oppressions and are fleetingly sorry, but to him each such instance was a personal hurt. Although insistent upon high standards, he could overlook human foibles and frailty. When it did become necessary to reprove a subordinate he could point out the correct way with a parable, so that one would hardly know that a reprimand had been administered.

Inevitably he tried to carry too big a load, but when the pressure of his work allowed he enjoyed the opera or a good book. It will probably come as a surprise to many who knew him that he wrote poetry. Each birthday, anniversary, or other

special occasion would bring with it one of his humorous jingles, his own teasing way of congratulating friends and family.

Dr. Palkin was a member of Sigma Xi, The American Chemical Society, the American Association for the Advancement of Science, and The Washington Academy of Sciences, and he was a Fellow of the Institute of Chemists.

He is survived by his widow, Rose Epstein Palkin; a daughter, Harriet Barbara Schwartz; a sister, Miss Dora Palkin; and a brother, Dr. J. R. Palkin, all of Washington, D. C.; and by a legion of friends and associates.

C. A. BROWNE

I. A. GOLDBLATT

<sup>1</sup> Bull Bur Chem, **162**, p. 219

<sup>2</sup> *This Journal*, **1**, 343 (1915-16).

<sup>3</sup> *J. Ind. Eng. Chem.*, **6**, 751 (1914).

<sup>4</sup> *Ibid.*, **7**, 519 (1915).

<sup>5</sup> *J. Am. Chem. Soc.*, **38**, 2166 (1916)

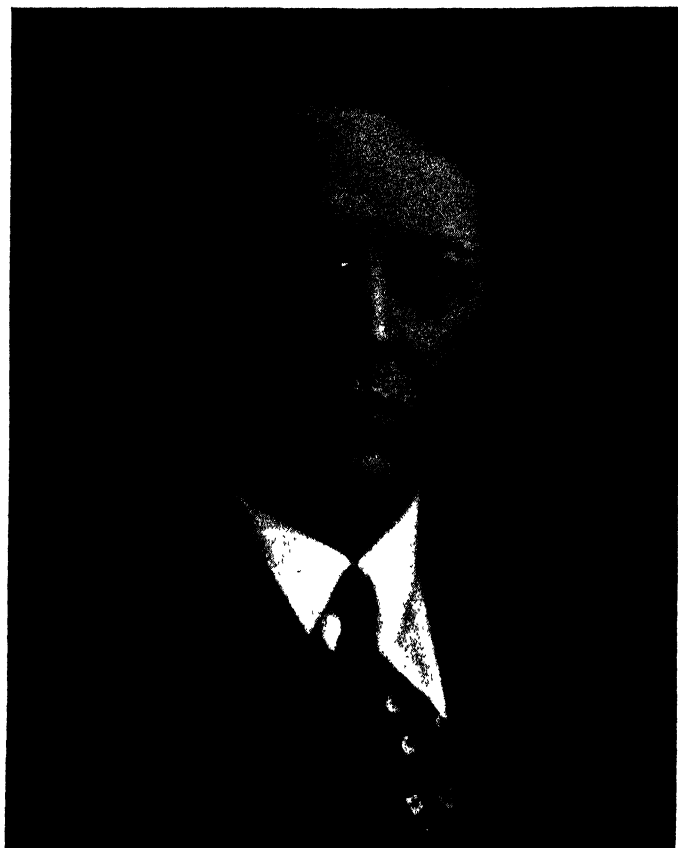
<sup>6</sup> *This Journal*, **7**, 14 (1923-4), **8**, 30 (1924-5)

<sup>7</sup> *Ibid.*, **8**, 541 (1924-5)

<sup>8</sup> *Ibid.*, **9**, 326 (1926)

<sup>9</sup> *Ibid.*, **10**, 130 (1927)

<sup>10</sup> *Ibid.*, **18**, 466 (1935)



DR. EARL PERRY CLARK, 1892-1943

## EARL PERRY CLARK

The recent death of Dr. Earl P. Clark, a senior chemist of the Division of Insecticide Investigations of the Bureau of Entomology and Plant Quarantine, removed from the ranks of the Association of Official Agricultural Chemists one of its most efficient members. Declining health had obliged him to retire from the active duties of his position in May 1943, but the hoped-for recovery failed to ensue. After a long wasting illness he passed away at his home in Washington on November 7, 1943.

Clark was born at Portland, Oregon, on August 25, 1892. His first chemical experience was acquired while still a boy in the pharmaceutical laboratory of the Spokane Drug Company of Spokane, Washington. His duties here consisted in helping to prepare U. S. Pharmacopoeia and National Formulary products and in doing some analytical work for which even at this early period he showed a remarkable aptitude. In 1912 he began an undergraduate course in chemistry at Washington State College but left in 1915 to become a chemist aid in the Carbohydrate Division of the Bureau of Chemistry. He acquired here his first experience in preparing the rarer sugars and their derivatives. In May 1916, Clark accepted a fellowship at the Rockefeller Institute for Medical Research, where for the next four years he conducted investigations on carbohydrates, nucleic acids, and other physiological products. His desire for varied activity prompted him in 1920 to become an Associate Technologist at the National Bureau of Standards, where he continued his research work on the rarer sugars. He resigned this position in 1922 to take graduate work in chemistry at the State University of Iowa, from which he received the degree of M.S. in 1923 and of Ph.D. in 1924.

After acquiring his doctorate Clark worked two years as research chemist in the Department of Biochemistry at the University of Alberta, Edmonton, Canada, where he conducted investigations on the hormones of the parathyroid gland. In December 1926 he accepted a fellowship at the Bureau of Chemistry in Washington, under a grant of the Interstate Cottonseed Crushers Association, for the purpose of investigating the chemistry of gossypol— a toxic organic constituent of cottonseed. An attractive opportunity for studying the chemistry of other physiologically active plant substances induced him in July 1929 to join the staff of the Division of Insecticide Investigations of the Bureau of Chemistry, where he remained until 1934, when this Division was transferred to the Bureau of Entomology and Plant Quarantine. He remained until his retirement with this Bureau, where he continued his investigations on naturally occurring insecticidal substances.

Clark's first work for the Association of Official Agricultural Chemists was a paper presented at its 1931 meeting on "The Viebock and Schwappach Method for the Determination of Methoxyl and Ethoxyl Groups."<sup>1</sup> This contribution was an outgrowth of his extensive laboratory experience in determining and orienting the methoxyl groups in deguelin, tephrosin, rotenone, and their derivatives, on the chemical constitution of which he was then busily engaged. In his examination of these and other related compounds, where only minute quantities of material were available, Clark made extensive use of micro- and semi-microanalytical methods. His exploration of this field led to the presentation at the Association's 1932 meeting of an important paper on "Microanalytical Methods."<sup>2</sup> This contribution so impressed the meeting that he was made Referee on Microchemical Methods— an appointment that he continued to fill during the remainder of his life. His additional contributions for the Association to this field are papers on "Semi-micro Determination of Carbon and Nitrogen,"<sup>3</sup> "Semi-micro Determination of Nitrogen by the Dumas Method,"<sup>4</sup> "Semi-micro Determination of Halogens in Organic Com-

pounds,"<sup>6</sup> "Microchemical Alkoxy Determinations,"<sup>6</sup> "Microchemical Kjeldahl Nitrogen Determinations,"<sup>7</sup> and various other miscellaneous referee reports.<sup>8</sup> A paper by Clark and F. Hillig, "Concerning the Dyer Method for the Identification and Determination of Volatile Fatty Acids,"<sup>9</sup> was read at the 1937 meeting.

In his improvement of the delicate microanalytical methods Clark devised a large number of pieces of microapparatus in the construction of which he showed great dexterity and manipulative skill. He blew his own glassware, made his own microcombustion train, and improved the mountings of his microbalance. Descriptions of new forms of apparatus are included in many of Clark's papers. More rarely he devoted a special note, or short article, to the account of some new laboratory device as in his papers on "An Electrically Heated Sand Bath"<sup>10</sup> and "An Electrically Heated Abderhalden Dryer."<sup>11</sup> Clark's long work table, with its crowded assemblage of apparatus for percolation, distillation, combustion, melting point, molecular weight, and other miscellaneous operations, gave his laboratory a touch of the atmosphere of a Teniers painting.

With the exception of occasional book reviews for its *Journal*, Clark's work for the Association was limited to descriptions of new analytical methods and improvements in laboratory apparatus. These subjects represent, however, only a small fraction of his total agricultural chemical contributions. He is best known for his work on the chemical composition and properties of various organic compounds (either toxic or having a possible insecticidal value) that occur in several classes of widely distributed plants. Clark's seven papers on gossypol, five papers on toxicarol, four papers on deguelin, three papers on tephrosin, and his series of contributions on the composition and toxicity to insects of the natural bitter substances, quassin, neoquassin, tenulin, helenalin, picro-toxin, picrosmin, etc., published chiefly in the *Journal of the American Chemical Society*, constitute the most important of his publications in this field. The last of Clark's publications is a posthumous book, "Semi-micro Quantitative Organic Analysis," with 134 pages and 31 figures, published by the Academic Press Inc., 125 East 23rd Street, New York. It is unfortunate that he did not live to see a copy of this last work of his productive pen.

In the prime of life Clark was a man of strong physique. One of his chief recreations was archery, in which he became highly proficient. With his accustomed attention to detail and manual dexterity he made his own implements—bows from personally selected and properly cured specimens of yew and Osage orange woods and arrows from carefully turned sticks from the pine, cedar, and fir trees. He was well versed in the history and extensive literature of archery and took an active part in the tournaments of this sport. He was a member of the Potomac Archers Club.

For mental relaxation Clark frequently turned to the solace of music. While still a young man his participation in choral activities stimulated an interest in the opera. Serious study developed this interest into a keen appreciation of the works of the great composers, particularly those of Wagner, which were among his favorites.

Besides the Association of Official Agricultural Chemists, Clark was a member of the American Chemical Society, the American Association for the Advancement of Science, Association of Biological Chemists, and Sigma Xi. He was married on November 25, 1920, to Helen May Vreeland, to whom the sympathy of his many friends in the Association is extended.

C. A. BROWNE

<sup>1</sup> *This Journal*, 15, 136-40 (1932).

<sup>2</sup> *Ibid.*, 16, 255-60 (1933).

<sup>3</sup> *Ibid.*, 414-18.

<sup>4</sup> *Ibid.*, 575-80.

<sup>5</sup> *Ibid.*, 17, 483-7 (1934).

<sup>6</sup> *Ibid.*, 22, 632-4 (1939).

<sup>7</sup> *Ibid.*, 24, 641-7 (1941).

<sup>8</sup> *Ibid.*, 17, 382-3 (1934); 18, 439 (1935).

<sup>9</sup> *Ibid.*, 21, 684-8 (1938).

<sup>10</sup> *Ibid.*, 16, 418-20 (1933).

<sup>11</sup> *J. Am. Chem. Soc.*, 20, 306 (1928).

## REPORT ON FILL OF CONTAINER METHODS FOR FOODS, DRUGS, AND COSMETICS

By S. C. ROWE (Food and Drug Administration, Federal Security  
Agency, Washington, D. C.), *Referee*

At the 1941 meeting of the Association methods for measuring the fill of container of foods, drugs, and cosmetics were presented in a paper which was later published in *This Journal*, 25, 248. All these methods, which include volume determination of liquids, semi-solids, and solids (free-flowing and non-flowing), in various types of containers, have been used for at least 2 years in law enforcement work, and a few of them, such as the specific gravity net weight method for determining volume of liquids, have been used for many years. Each method has been developed by more than one chemist, and necessary modifications have been made from time to time. While samples have not been sent out for collaborative study, it is believed that sufficient work has been done to warrant their adoption as tentative methods by the Association, and it is so recommended.\* It is also recommended that they be studied collaboratively with a view to their adoption as official methods at a later date.

## REPORT ON COLORING MATTER IN FOODS

By C. F. JABLONSKI (Food and Drug Administration, Federal  
Security Agency, New York, N. Y.), *Referee*

Owing to adverse conditions, the Referee was unable to devote much time to the study of the problems recommended by the Association.

However, a rather comprehensive study was made of the detection of minute amounts of FD&C Yellow No. 5 (tartrazine) in alimentary paste. This method is based on suggestions made by a member of the chemical staff of the New York City Board of Health laboratory. Many modifications of the suggested method were necessary to overcome the usual difficulties encountered in alimentary paste products. As a result the following method is suggested.

Place 800 ml. of cold distilled water and 5 ml. of strong ammonia water into a liter Erlenmeyer flask and add 200 grams of the unground sample. Stopper flask and shake at intervals for 3-4 hours, usually sufficient time to disintegrate the material. Use a glass rod to dislodge material caking on bottom. Centrifuge and decant the clear supernatant liquid into a liter flask, add a solution of 50 grams of  $MgSO_4$  dissolved in 100 ml. of water, 10 ml. of 12% silicotungstic acid and 10 ml. of concentrated HCl, shake well, and stand for 1 hour. (This treatment will precipitate almost all protein matter.)

Centrifuge and decant the clear solution into a liter casserole. (In the presence of a minute amount of color, the solution will appear to be almost colorless.) Place into

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 27, 64 (1944)

the liquid four pieces of washed wool (about 2" X 2") and heat on steam bath until the coloring matter is absorbed by the fiber. (Under ordinary conditions, this will be accomplished by concentration of the solution to half the original volume.) Remove wool and wash with water to free from adhering foreign matter. Transfer wool to a 125 ml. casserole, add about 25 ml. of water and a few drops of strong ammonia, and warm over steam bath to remove the color from cloth. Discard wool. Evaporate the alkaline color solution to dryness. To the residue add 25 ml. of water to dissolve the dye and filter. (The amount of coloring matter recovered is approximately 40% of total.) Divide the solution into two portions; one of 15 ml., the other 10 ml. Place the larger portion into a small (50 ml.) casserole, acidify slightly with HCl, and add a small piece of washed wool to absorb the dye. Spot the dyed wool for tartrazine, comparing the color with a standard of approximately the same intensity.

To the smaller portion of the solution, add 2 drops of HCl and an excess of bromine water. If a precipitate forms at this stage owing to the amount of soluble protein matter present, centrifuge or filter off this precipitate to obtain better results on coupling. Destroy excess of bromine with about 1 ml. of saturated aqueous hydrazine sulfate solution and couple immediately with 1 drop of 1% alcoholic alpha naphthol in 5 ml. of 10% aqueous Na<sub>2</sub>CO<sub>3</sub> solution. The formation of orange or pink coloring indicates presence of tartrazine FD&C Yellow No. 5.

#### RECOMMENDATIONS\*

The Referee, therefore, recommends—

- (1) That collaborative work on the proposed method be undertaken.
- (2) That investigational work be conducted on the quantitative separation and estimation of FD&C Yellow No. 5 (tartrazine) and FD&C Yellow No. 6 (sunset yellow).
- (3) That investigational work be conducted on the quantitative separation and estimation of FD&C Green No. 2 (light green SF yellowish), FD&C Green No. 3 (fast green FCF), and FD&C Blue No. 1 (brilliant blue FCF).
- (4) That investigational work be undertaken on the quantitative separation and estimation of FD&C Yellow No. 3 (yellow AB), FD&C Yellow No. 4 (yellow OB), FD&C Orange No. 2 (Orange SS), and FD&C Red No. 32 (oil red XO).
- (5) That collaborative work on analytical methods for coal-tar color certifiable for use in foods be conducted.

#### REPORT ON LACTOSE IN MILK

By E. R. GARRISON (Agricultural Experiment Station,  
Columbia, Mo.), *Associate Referee*

Previous reports (*This Journal*, **22**, 489; **25**, 603) have shown that the volume of protein and fat in 65.8 grams (2 N. weight) of milk varies with the composition of the sample and that inadequate allowance for the volume of precipitate in the dilution of the milk is an important source

\* For report of Subcommittee C and action by the Association, see *This Journal*, **27**, 60 (1944).

of error in the optical determination of lactose by the A.O.A.C. method (*Methods of Analysis, A.O.A.C.*, 1940, 271, 16). The double dilution method,<sup>1</sup> which theoretically accurately corrects for the volume of precipitate in each sample, gave lactose values for 59 samples of milk that averaged 0.22 per cent lower than similar values obtained by the A.O.A.C. dilution method. For these samples the average volume of precipitate in 65.8 grams of milk was 5.2 ml., which is twice as much as the 2.6 ml. allowed for the precipitate in the A.O.A.C. dilution method.

Collaborative work, as recommended by Committee C in 1941 (*This Journal*, 25, 68), on the double dilution method for correction for the volume of precipitate in the optical determination of lactose in milk was done during the past year by four investigators. Several cans of evaporated milk from the same batch of milk were obtained from a condensery by the Associate Referee, and one can was sent to each collaborator with detailed instructions for making the optical determination of lactose by the double dilution and A.O.A.C. dilution methods. Each collaborator was also requested to obtain 9 samples of fluid milk of varying fat percentages from local sources and analyze each sample for lactose by each of the above procedures.

The table shows the collaborative results obtained. The remarks by the collaborators are as follows:

*S. F. Hinckle.*—It was difficult to fill the flasks to mark accurately because of the foam produced. The percentage differences of lactose found by these two methods do not seem to vary with the amount of precipitate. It was noticed in all instances that the difference was in the same direction, specifically indicating that the 2.6 ml. allowed for the volume of the precipitate in the A.O.A.C. method is inadequate.

*Carl S. Ferguson.*—From the work that I have been able to do, I believe that the double dilution method is preferable and therefore recommend its adoption.

The data secured by the collaborators are in keeping with the results previously obtained by the Associate Referee and referred to at the beginning of this report. The A.O.A.C. method gave lactose values that varied from —.01 to .50 and averaged .17 per cent higher than the lactose values obtained by the double dilution method on 31 samples of fluid milk and one sample of evaporated milk. Three of the collaborators obtained essentially the same difference in lactose values by the two dilution methods with the sample of evaporated milk, namely .12, .12, and .15 per cent, while the other collaborator secured essentially the same result by each dilution method with this milk sample. The amount of lactose found in the evaporated milk by the different investigators varied from 4.40 to 4.09 per cent by the A.O.A.C. dilution method and from 4.28 to 4.10 per cent by the double dilution procedure. The reason for the rather wide variation in the results obtained is unknown but experimental error is no doubt an important factor. Important sources of error in the optical

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<sup>1</sup> *Analyst*, 21, 182 (1896); *This Journal*, 25, 608 (1942)



method that cannot be entirely eliminated are (1) the incorporation of air into the curd during the addition of the reagents and (2) the formation of foam, which prevents accurate filling of the flasks to the mark. The error in the optical procedure that exists because of under dilution in the

*Lactose values obtained by A.O.A.C. and double dilution methods*

COLLABORATOR	SAMPLE NO	TYPE OF MILK	A. O. A. C. METHOD	DOUBLE DILUTION METHOD	DIFFERENCE
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
S. F. Hinckle	--	Evaporated	4.40	4.28	.12
Hershey Chocolate Corp.	1	Mixed herd	5.20	5.03	.17
Hershey, Pa.	2	Mixed herd	5.03	4.82	.21
	3	Mixed herd	5.05	4.97	.08
	4	Guernsey	5.28	5.14	.14
	5	Guernsey	5.08	4.98	.10
	6	Guernsey	5.38	5.10	.28
	7	Holstein	5.14	4.99	.15
	8	Holstein	5.25	5.10	.15
	9	Holstein	5.16	4.92	.24
C. E. Hynds	-	Evaporated	4.32	4.20	.12
State Food Lab	10	Market	4.90	4.70	.20
Albany 1, N. Y.	11	Market	4.90	4.65	.25
	12	Market	4.60	4.40	.20
	13	Herd—high fat	5.10	4.95	.15
	14	Herd—high fat	5.00	4.85	.15
	15	Herd—low fat	3.80	3.70	.10
	16	Herd—low fat	3.95	3.75	.20
E. R. Garrison	—	Evaporated	4.33	4.18	.15
Agr. Exp. Sta.	17	Mixed herd	4.93	4.83	.10
Columbia, Mo.	18	Mixed herd	4.89	4.68	.21
	19	Mixed herd	4.83	4.73	.10
	20	Jersey	5.20	4.88	.32
	21	Jersey	5.18	5.04	.14
	22	Jersey	5.08	4.78	.30
	23	Holstein	4.99	4.86	.13
	24	Holstein	4.90	4.71	.19
	25	Holstein	4.85	4.73	.12
Carl S. Ferguson	-	Evaporated	4.09	4.10	.01
Dept. Pub. Health	26	Guernsey	4.65	4.50	.15
Boston, Mass.	27	Guernsey	4.90	4.40	.50
	28	Guernsey	4.95	4.73	.22
	29	Holstein	4.58	4.35	.23
	30	Holstein	4.80	4.78	.02
	31	Holstein	5.25	5.10	.15
		Average	4.85	4.68	.17

A.O.A.C. method can be eliminated by using the double dilution method, or the error in the A.O.A.C. method can be largely eliminated by allowing 5 ml. for precipitate instead of the 2.6 ml. now allowed in this procedure.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the double dilution method for correction of the volume of precipitate in the optical determination of lactose in milk be adopted as official, first action.

(2) That the method be amended to provide that the sample of milk for the optical determination of lactose be weighed into the flask instead of measured as is now specified on page 271, *Methods of Analysis A.O.A.C.*, 1940.

#### REPORT ON DRIED AND SKIM MILK (LACTIC ACID)

By FRED HILLIG (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

At the 1941 meeting of the Association (*This Journal*, 25, 602) it was recommended that the method for the determination of lactic acid in milk and milk products (*Ibid.*, 20, 134) be made tentative.

In order to study the application of the method when volatile acids (formic, acetic, and butyric acids) are present, samples containing these acids and also lactic acid in varying quantities unknown to collaborators were submitted. Owing to the fact that this work was conducted in connection with the determination of volatile acids it seemed logical to keep the results together since this phase of the investigation is applicable to the determination of these acids in a number of food products in which decomposition has taken place. Therefore, the results will be presented in the report on Volatile Acids in Fish and Fish Products (see p. 237). It will be noticed that the results with few exceptions are quite satisfactory.

It is recommended† that the study of the method for the determination of lactic acid be continued with a view to its adoption as official.

#### REPORT ON FISH AND OTHER MARINE PRODUCTS

By H. D. GRIGSBY (Food and Drug Administration, Federal Security Agency, Philadelphia, Pa.), *Referee*

Owing to war conditions, the only report submitted since the 1941 meeting was that of Associate Referee Manuel Tubis (*This Journal*, 26, 226).

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 27, 83, 88 (1944).

† For report of Subcommittee C and action by the Association, see *This Journal*, 27, 83 (1944).

## RECOMMENDATIONS\*

It is recommended—

- (1) That the vacuum oven method presented by the associate referee for the determination of moisture in fish be made tentative,
- (2) That the modification of the Veshchezerov method for the rapid determination of moisture be made tentative,
- (3) That the Stansby-Lemon method<sup>1</sup> for the determination of fat be made tentative,
- (4) That the Stansby-Lemon rapid semiquantitative method for the determination of fat be studied collaboratively,
- (5) That the Kaye-Leibner-Connor method<sup>2</sup> for the determination of moisture and fat be studied collaboratively,
- (6) That the methods for the determination of volatile acids and formic acid in canned salmon and tuna fish (*This Journal*, 21, 684, 688) be studied collaboratively,
- (7) That methods for the determination of volatile bases in fish be studied.

The details of the methods recommended in (1), (2), and (3) follow:

## MOISTURE

## PREPARATION OF SAMPLE—TENTATIVE

Prepare the sample according to the type as directed under 2 (*Methods of Analysis*, A.O.A.C., 1940, 317) and keep the ground material in a sealed jar.

If the jar has been chilled, allow the samples to come to room temperature and shake the jar so that any separated moisture will be removed by the fish. Open the jar and quickly stir the contents, thoroughly contacting the sides and lid so as to incorporate any separated liquid; weigh approximately 5 grams into a dish, prepared as directed below, cover quickly, and finish the weighing.

*Preparation of dishes.*—Fit a lead foil or other foil dish into an aluminum drying dish approximately 70 mm. in diameter and 33 mm. high and provided with a well fitting cover. Place in the foil dish approximately 20 grams of clean, fat-free, dry sand and a 1½ inch glass rod with a flattened lower end so that its upper end remains clean. Dry the unit at 100°–110°C. for 1 hour, cool in desiccator, and weigh.

*Vacuum Oven Method*

Dry the samples, prepared and weighed as directed above, in a vacuum oven at 98–100° at less than 25 mm. pressure to constant weight (about 5 hours). Admit dry air into the oven until atmospheric pressure is reached, and immediately tighten cover; transfer sample to a desiccator, cool, and weigh when room temperature is attained. Reheat, and reweigh until loss in weight is 1 mg. or less.

*Rapid Method*

(Results closely approximate those obtained by the vacuum method. Not to be used for subsequent fat extraction.)

Dry the samples at 70°C. ( $\pm 5^\circ$ ) for 1.5 hours, and then at 130°C. ( $\pm 5^\circ$ ) for 1 hour, cool in a desiccator, and weigh.

\* For report of Subcommittee C and action by the Association, see *This Journal*, 27, 62 (1944).

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 9, 341 (1937).

<sup>2</sup> *J. Biol. Chem.*, 132, 195 (1940).

## CRUDE FAT—ETHER EXTRACT

## QUANTITATIVE METHOD

Into a previously dried thimble in a stoppered weighing tube weigh approximately 15 grams, to the nearest centigram, of the well mixed sample. Extract for 16 hours in a continuous extractor, using acetone and replacing the solvent and flask with another flask containing fresh acetone after the first 2 hours. Remove the acetone and most of the water by heating on a steam bath. Place the flasks in a vacuum desiccator over freshly boiled  $\text{H}_2\text{SO}_4$ , evacuate, and allow to stand overnight or until most of the water has been removed, as shown by the cessation of foaming and the disappearance of any water droplets from the sides of the flasks. Dissolve the fat in the flask with 35 ml. of anhydrous ethyl ether, mixing the contents well so as to remove any fat held by the gummy material, and filter through a medium-porosity, sintered-glass funnel into a weighed "fat" or "beaker flask," using a similar flask as a counterpoise. Rinse both flasks with each portion of ether, passing them through the funnel until the washings are colorless. Evaporate the ether spontaneously or on a warm surface and when the odor of ether is no longer detectable, heat the flasks in an oven at  $100^{\circ}$ – $105^{\circ}\text{C}$ . for 1 hour with the counterpoise. Cool in air and weigh. Reheat for a second hour, or until loss in weight does not exceed 5 mg., and reweigh.

## REPORT ON VOLATILE ACIDS IN FISH AND FISH PRODUCTS

By FRED HILLIG (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The Associate Referee has published several papers<sup>1,2,3,4</sup> on the determination of volatile acids in fish and fish products, wherein it was pointed out that such a determination offered a means of detecting decomposition. An apparatus that was found suitable for the work and that could be easily constructed from material in the laboratory was described.<sup>1</sup> The method did not determine the total volatile acid content of a product nor did it differentiate between the volatile acids present. Formic acid was determined in the distillate by the reduction method. The results were expressed as volatile acid number and formic acid number.

In a later paper<sup>5</sup> a method was proposed by which individual volatile acids present could be quantitatively determined. The method utilized the apparatus previously described.<sup>1</sup> This method has now been subjected to an extensive collaborative study.

In the proposed procedure distillation data on volatile acids of known purity must be obtained on each apparatus. These distillation data are necessary for use in setting up the simultaneous equations that are employed in computing the kind and quantity of volatile acid or acids present. The theory and complete description of the method have been published.<sup>5</sup>

<sup>1</sup> *This Journal*, 21, 684 (1938).

<sup>2</sup> *Ibid.*, 698.

<sup>3</sup> *Ibid.*, 22, 116 (1939).

<sup>4</sup> *Ibid.*, 414.

<sup>5</sup> *Ibid.*, 25, 176 (1942).

TABLE 1.—*Standardization of distillation apparatus*  
(Per cent acid distilled in one 50 ml. and three 200 ml. portions of distillate.)

COLLABORATOR	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<b>Formic acid</b>																				
50 T1	11.3	10.7	11.0	10.8	10.5	11.4	11.2	10.5	11.0	10.7	11.0	11.2	11.2	10.7	10.5	11.5	9.9	11.3	11.4	11.0
200 T2	33.8	33.2	33.4	32.8	33.0	33.8	33.3	32.1	32.7	32.2	33.4	33.9	33.4	32.6	32.0	34.4	31.5	34.4	34.2	33.2
200 T3	20.9	20.8	20.3	20.4	20.6	20.9	20.5	20.6	19.9	20.1	20.3	20.7	20.7	20.8	21.7	20.6	20.6	21.1	20.7	20.7
200 T4	12.9	12.9	12.8	12.8	13.3	13.2	12.8	13.2	12.8	12.8	12.5	12.7	12.9	12.8	13.9	12.8	12.8	12.9	12.6	13.2
T2/T1	2.99	3.10	3.04	3.04	3.14	2.96	2.97	3.05	2.97	3.02	3.04	3.03	2.99	3.04	3.05	2.98	3.23	3.04	3.00	3.01
<b>Acetic Acid</b>																				
50 T1	19.3	18.7	18.0	18.0	17.8	18.7	18.8	17.7	18.2	18.5	18.8	18.9	18.8	17.4	17.7	19.2	18.4	18.5	19.3	18.3
200 T2	46.5	46.4	45.6	45.5	44.2	45.2	44.9	44.2	43.1	44.9	45.9	46.1	46.2	44.2	45.0	45.7	47.6	45.7	46.3	46.0
200 T3	19.6	19.9	20.0	20.5	19.9	20.0	19.8	20.5	19.9	19.8	19.5	20.0	19.4	20.1	20.5	19.3	20.6	20.1	20.2	20.6
200 T4	8.3	8.6	8.8	9.1	9.4	8.7	8.9	9.5	9.4	9.0	8.6	8.7	8.4	9.1	9.5	8.8	8.5	8.3	8.5	8.7
T2/T1	2.41	2.48	2.53	2.53	2.43	2.42	2.39	2.50	2.36	2.43	2.44	2.44	2.45	2.53	2.55	2.37	2.59	2.47	2.40	2.51
<b>Propionic Acid</b>																				
50 T1	33.9	33.7	31.7	31.0	32.1	33.2	32.8	31.9	32.9	32.7	32.3	32.3	33.5	32.3	31.9	32.4	30.1	33.0	33.6	32.6
200 T2	53.3	53.1	54.0	53.0	53.4	52.4	52.8	52.9	54.5	53.0	52.3	52.8	53.5	53.0	53.1	52.6	52.9	52.9	52.9	52.3
200 T3	3.4	3.3	3.7	3.8	3.8	3.5	3.9	4.3	3.9	3.9	4.3	3.6	3.6	4.2	3.7	3.7	3.3	3.3	3.4	4.1
200 T4	0.3	0.4	0.4	0.3	0.4	0.3	0.6	0.5	0.4	0.4	0.5	0.5	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.4
T2/T1	0.97	0.97	1.00	0.99	0.99	0.98	0.99	1.05	1.01	1.00	1.04	1.05	0.99	1.05	1.00	1.00	1.00	0.98	0.95	1.02
<b>Butyric Acid</b>																				
50 T1	48.9	49.1	47.8	48.0	47.8	48.6	47.9	46.1	47.7	47.7	46.7	46.5	48.1	46.4	47.6	47.7	47.8	48.2	49.4	47.2
200 T2	47.4	47.7	47.7	47.6	47.3	47.5	47.4	48.5	48.0	47.6	48.8	48.5	47.6	48.8	47.8	47.9	48.0	47.3	46.8	48.3
200 T3	3.4	3.3	3.7	3.8	3.8	3.5	3.9	4.3	3.9	3.9	4.3	3.6	3.6	4.2	3.7	3.7	3.3	3.3	3.4	4.1
200 T4	0.3	0.4	0.4	0.3	0.4	0.3	0.6	0.5	0.4	0.4	0.5	0.5	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.4
T2/T1	0.97	0.97	1.00	0.99	0.99	0.98	0.99	1.05	1.01	1.00	1.04	1.05	0.99	1.05	1.00	1.00	1.00	0.98	0.95	1.02
<b>Iso-Butyric Acid</b>																				
50 T1	62.2	62.6	62.0	61.4	61.5	61.8	60.5	60.2	61.6	61.6	59.8	59.8	61.3	57.9	60.8	61.7	58.5	61.7	62.7	—
200 T2	37.0	36.5	36.5	36.4	36.4	37.0	37.9	37.8	37.1	36.6	38.5	38.5	37.2	39.6	37.6	38.8	39.4	38.3	36.3	—
200 T3	0.8	0.8	1.1	0.9	1.0	0.9	1.5	1.2	1.3	1.1	1.3	1.3	1.0	0.9	1.0	1.0	1.3	0.9	1.0	—
200 T4	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	—
T2/T1	0.59	0.58	0.59	0.59	0.59	0.60	0.63	0.63	0.60	0.59	0.64	0.64	0.61	0.68	0.62	0.60	0.67	0.59	0.58	—



Volatile acid solutions (the purity of which was established following the procedure previously published<sup>6</sup>), consisting of approximately 0.1 *N* formic, acetic, propionic, butyric, and isobutyric acids, to be used in securing the distillation data required on each individual distillation outfit were submitted to collaborators. The collaborators were instructed to transfer 50 ml. of the formic acid solution to the distillation flask of the distillation outfit, make to 150 ml. with boiled distilled water, and distil under the conditions previously specified,<sup>5</sup> collecting one 50 ml. and three 200 ml. portions of distillate, and titrating each portion separately. They were then directed to compute the percentage of acid distilled in each portion of distillate and to submit the figures to the Associate Referee. This procedure was to be followed for each acid. In Table 1 these data are presented.

It will be noticed that in spite of the fact that different distillation outfits were used by different collaborators very close agreement was obtained. It is emphasized at this point that since each outfit will give distillation data peculiar to that outfit, these data must be obtained on each distillation outfit in order that the proper simultaneous equations may be set up and solved.

The next step in the study of the method was to submit solutions containing volatile acids in varying quantities unknown to the collaborators. The results are summarized in Table 2.

A study of the table will show that only in a few instances were results obtained that were somewhat out of line with the quantity of acid present. When one considers the nature of the determination being made, the results appear to be very satisfactory.

Since the ground work for the application of the method to fish and fish products (as well as other food products) has now been laid, it is recommended that the work be continued.

The Associate Referee wishes to thank the following collaborators, all members of the Food and Drug Administration, for their splendid cooperation. A minimum of two weeks' time was spent by each collaborator in gathering the data submitted in this report.

Duncan H. Holaday, L. H. McRoberts—San Francisco Station  
 William Horwitz—Minneapolis Station  
 F. J. McNall, Iman Schurman—Cincinnati Station  
 Paul Mills, C. C. Cooley—Seattle Station  
 Leslie W. Ferris—Buffalo Station  
 C. A. Wood, G. Kirsten—New York Station  
 M. Tubis—Philadelphia Station  
 H. I. Macomber—Baltimore Station  
 C. D. Schiffman—Atlanta Station  
 R. D. Stanley—Chicago Station  
 G. M. Johnson—St. Louis Station  
 L. L. Ramsey, L. M. Beacham, Mae Carstensen—Washington, D. C.

<sup>6</sup> *This Journal*, 26, 198 (1943).

## REPORT ON METALS IN FOODS

By H. J. WICHMANN (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

### ARSENIC AND ANTIMONY

The Associate Referee on Arsenic and Antimony has made no report since the last annual meeting owing to other demands on his time. There has been no great Association interest in this field of late, but in the post-war years it is expected that the subject will need to be taken up again.

While there has been no great progress inside the Association, others have not forgotten the subject of arsenic. The Referee has been privileged to read a manuscript on an ultra micro Gutzeit method that will be published soon. This method is intended for the determination of approximately one microgram. It incorporates a number of new ideas, one being the evolution of hydrogen and arsine in a partial vacuum, the arsine being caught on a very small paper disc, which is then processed and compared photometrically with photographic standards. The accuracy obtained with small quantities of arsenic seems remarkable. The partial-vacuum idea appears to be well worth looking into. This paper also poses the theory that fresh blood, urine, and expired air contain a "volatile" form of arsenic. We have long wrestled with refractory forms of arsenic, but here is an instance of just the opposite phenomenon. This poses the question: Will nitric acid oxidize and render non-volatile this volatile arsenic in fresh biological material? It will be necessary to obtain some answer to this question.

### COPPER

The Associate Referee on Copper made no report owing to a mishap in the preparation of the samples for the collaborators. The work, however, should be continued. It is hoped that a tentative carbamate method for copper can be adopted in time for the next revision of *Methods of Analysis, A.O.A.C.* In the meantime interest in all dithizone methods for the determination of copper is maintained. The Referee calls attention to a recent paper by Bendix and Grabenstetter<sup>1</sup> on this subject. It is his recommendation that the study of micro methods for the determination of copper be continued, and that as soon as the carbamate method has been perfected studies on the "all dithizone method" be inaugurated.

### FLUORINE

The determination of fluorine has been an active project of the Association for a long time. Therefore both referees are pleased to be able to recommend a tentative general method for the determination of fluorine

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<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 15, 649 (1943).



in foods and a rapid one restricted to the determination of spray residue fluorine on apples and pears. The recommendation is based on collaborative work conducted during the last few years. The general method, based on the pioneer distillation method of Willard and Winter, has an accuracy of approximately one microgram in the micro range. The rapid method, based on the work of Kolthoff and Stansby,<sup>2</sup> dispenses with the distillation and the fluorine in strong hydrochloric acid is titrated with zirconium nitrate solution and purpurin as indicator. It uses the same solutions and stripping procedure as do the present rapid lead and arsenic methods for spray residue work. This should cut down the cost of fluorine analyses in the fruit industry materially. The zirconium-purpurin titration in strong acid is not as sensitive as the thorium-alizarin procedure of the general method, but it is sufficiently sensitive for the purpose intended, and the acid concentration minimizes the effect of most of the interfering elements found on fruit. The accuracy of the titration itself is of the order of .001-.002 grain per pound. Not all of the fluorine is dissolved from the fruit, but it is expected that a fluorine assay on apples or pears by the method proposed will recover 95 per cent of the amount present. The analytical error is therefore appreciable, but probably considerably less than the error of sampling.

The fluorine situation may be described as being in a healthy condition. The Referee believes, however, that work on fluorine methods should be continued and no opportunities for improvement missed. Because it might be a clue to new developments the Referee calls attention to the statement in the discussion of a recent paper published in the *Analyst*, 68, 301 (1943), that certain dyestuffs, particularly Solochrome Brilliant Blue BS, are superior to alizarin as a fluorine indicator. The lake is said to be blue, and the color change on titration with thorium was from yellow to blue. Moreover, it is claimed that the reaction between thorium, dye and fluorine is faster than the analagous alizarin reaction. The structural composition of this British dye is not known to the Referee, but he expects to receive a sample. When it is at hand some experiments will be started promptly.

#### LEAD

The report of the Associate Referee on Lead (*This Journal*, 26, 26), shows a large amount of work accomplished. The photometric method of detecting bismuth interference and the simultaneous determination of lead and bismuth are worth special mention. The Associate Referee's work on urine, especially the part dealing with possible errors in the dithizone isolation of lead from phosphatic biological material, should be of particular interest to biological and pharmacological chemists. This work may

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<sup>2</sup> Ind Eng. Chem., Anal. Ed., 6, 118 (1934).

have no direct connection with the determination of lead in foods, but it has, nevertheless, a direct bearing on the accuracy of our lead methods in general, and on the wisdom of including the sulfide separation as an alternative procedure for first isolating the lead in those cases where the extractive dithizone isolation may not be applicable, as in phosphatic products. In the Referee's opinion, the lead methods are now in a most enviable position with respect to accuracy and specificity.

The problems remaining for attention at this time appear to be few in number. It is recommended that the projects listed by the associate referee be given attention as soon as possible. Perhaps the time is approaching when we should allocate to the referees of the various product chapters those methods of sample preparation which best fit their particular project, and leave in the chapter on metals in foods only those parts concerning the actual determination of lead itself.

It is in order to point out that the determination of spray residues (arsenic, lead, and fluorine) on fruits and vegetables can now be made on different aliquots of *the same sample solution*. This is a notable advance in efficiency and economy when considered in connection with the small analytical error of these determinations. The next logical step is to bring the sampling error to approximately the same level as the analytical error. One way to do this is to increase the size of the sample. The size of sample specified in both the current arsenic and lead spray residue methods was a compromise between what was desirable and what was practical and expedient at the time they were devised. The Referee believes that the time has now come for the initiation of some studies designed to answer the problem of how to remove the spray residue from larger samples of fruits and vegetables before assay. He suggests that a small-scale washer on either the churn principle, or on that of a miniature commercial fruit washer, might remove the spray residue from a peck to a bushel of apples, with other products in proportion. Production of such an addition to laboratory equipment would be welcome at this time.

#### MERCURY

The Associate Referee on Mercury made no formal report this year; however, the Referee has his assurance that the photometric dithizone method described in the last published report has been used with satisfaction in thousands of mercury determinations in foods and more particularly in biological samples. The Association is therefore in possession of two dithizone mercury methods—one photometric, the other titrimetric. The Referee believes that two tentative methods for the determination of mercury are not necessary. It is recommended that the new associate referee study both methods with the final aim of adopting a single method, which might be either of these or a combination of both,

according to the requirements of different classes of materials. The Referee recommends that the lead and fluorine methods be taken as models for a mercury method that could be adopted as tentative in time for incorporation into the next edition of *Methods of Analysis*, A.O.A.C.

#### SELENIUM

The last two reports of the Associate Referee on Selenium (*This Journal*, 25, 403; 26, 346) contain on the whole satisfactory collaborative results. The statistical analysis indicates that the 1943 results are much better than those of the previous year. This may be largely due to a refinement in the description of the method that had been incorporated in the interim. The Referee concurs with the associate referee in recommending that the selenium method described in the last report be adopted as an official method (first action).

#### ZINC

The Associate Referee on Zinc and his associates have attacked the problem of the determination of zinc from a different, if not wholly original angle. To avoid possible losses of zinc during ashing operations, they revert to a wet oxidation. They thus counterbalance opportunities for losses against possible contamination from the glass itself or from a greater consumption of reagents. The final choice of sample preparation procedure will be a matter of judgment and experience. They also go back to older methods for isolation of the zinc by precipitating the acid-insoluble metal sulfides at the start. It is apparent from the results that they have been able to accomplish a complete preliminary separation of micro quantities of lead and cadmium from the zinc. This is of some importance because the close relationship of cadmium to zinc has made previous efforts to remove it by hydrogen-ion control, competitive complex formation, or buffer action, a matter of some difficulty. Removal of cobalt and nickel is effected by non-aqueous solvent extraction of organic complexes of these metals that do not interfere with dithizone extraction of the zinc. This completes the isolation of the zinc. The extractive determination of the zinc from alkaline solutions then follows established methods. Experiments on 10 grams of sample produced recoveries of 97 to 105 per cent. Since the sample plus reagent blank amounted to 2.18 p.p.m. the actual zinc determined must have been in excess of 20 micrograms.

The Referee was interested in the 10 p.p.m. of zinc found in Pyrex glassware and the approximately 0.25 microgram of zinc found in blank determinations. These quantities of zinc will place a limit on the lowest quantity determinable from alkaline solutions without undesirable percentage errors. Contamination from all sources must be reduced to the lowest possible quantity and losses must be severely limited, if accurate determinations are to be made on quantities as low as 1-5 micrograms.

This may not be so significant on food products, but no doubt will be important on some biological materials. The Referee recommends that the study of zinc determinations be continued and that some work be done on the smaller amounts of zinc, as well as on the dithizone determinations of zinc from slightly acid solutions. In conclusion, the Referee desires to make a reference to the determination of zinc with the naphthol analogue of dithizone presented by Cholak, Hubbard, and Burkey.<sup>3</sup> No doubt some of the many possible analogues of dithizone may be of future service in the determination of many of the so-called dithizone metals.

#### HYDROCYANIC ACID

No report was made on the determination of hydrocyanic acid in foods. Since no report has been made for a number of years, it is recommended that this project be dropped until the determination of this fumigation residue again becomes of more general interest.

#### RECOMMENDATIONS\*

It is recommended—

- (1) That studies on the determination of arsenic and antimony be temporarily suspended, to be taken up again on the call of the Referee when the opportunity for such work again presents itself.
- (2) That work on the micro determination of copper be continued.
- (3) That the general method for the determination of fluorine in foods, and the rapid method restricted to the determination of spray-residue fluorine on apples and pears, as described by the associate referee, be adopted as tentative.
- (4) That studies on methods for lead in special products be continued.
- (5) That studies be begun on methods for removing spray residues (arsenic, fluorine, and lead) from larger samples of fruits and vegetables in connection with the rapid determination of such residues.
- (6) That an associate referee be appointed to study the two mercury methods before the Association, and to make a recommendation for a combination of the two or the elimination of one.
- (7) That the selenium method recommended by the associate referee be adopted as official (first action).
- (8) That work on a micro method for the determination of zinc be continued.
- (9) That work on the determination of hydrocyanic acid in foods be dropped.

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<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 15, 754 (1943).

\* For report of Subcommittee C and action by the Association, see *This Journal*, 27, 60, 90 (1944).

## REPORT ON FLUORINE

By P. A. CLIFFORD (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Results of 13 collaborators on 6 samples are tabulated and discussed in the Associate Referee's last report on fluorine (1). A further set of collaborative figures on these samples by W. Machle, E. Largent, and I. Ferneau of the Kettering Laboratory, received too late for inclusion in the report, fell in closely with the general averages. It was noted that collaborators obtained an average recovery of 99.2 per cent on Solution A (a pure solution of sodium fluoride, titrated directly without previous distillation) and only 93.0 per cent on Solution B (a similar solution with added sulfate and phosphate interference, in which it was necessary to isolate the fluorine before titration by means of a perchloric acid distillation). On the sample of port wine, with 5.00 p.p.m. added fluorine, a low average result was also obtained (94.2 per cent).

Such recoveries might indicate that the titration procedure used was satisfactory and that the low recoveries resulted from incomplete evolution of fluorine in the distillation. It now appears, however, that the titration itself was probably at fault, and re-examination of the thorium nitrate titration procedure in the light of the experience of many investigators reveals several possible sources of error. Most of these have been discussed in a previous report (2).

In this report it is pointed out that when dealing with somewhat smaller quantities of fluoride than did Willard and Winter (3), the original authors, the microtitration of the fluoride ion can not be compared, for instance, to the Mohr titration of chloride with silver nitrate solution and potassium chromate indicator. The alizarin-red indicator used in the microtitration of fluoride does not provide an abrupt color change at the equivalence point of thorium and fluorine, but color changes at the "end point" involve an equilibrium condition between fluorine and thorium and indicator and thorium. The end-point change is affected by many factors, among them being *pH*, salt concentration (especially of the alkali metals), the titration medium, concentration of indicator, temperature, etc. Still, it has been the usual custom to regard the titration of fluorine as a "precipitation-titration," and the fluorine equivalent of standard thorium solutions used almost invariably has been determined by titrating known quantities of fluoride (usually NaF, which is readily obtained pure) to an end point with alizarin under supposedly standard conditions.

Fluorine distills as fluosilicic acid under the conditions of the Willard-Winter distillation. After neutralization of a distillate and adjustment of *pH* to the proper point, the salt concentration of the resulting solution is not comparable to that of a solution of pure sodium fluoride containing an equivalent amount of fluoride. Hence, standardization of thorium

solution with sodium fluoride and determination of fluorine in a distillate with the resultant factor may not be a logical procedure for the micro-determination, especially as it is known that very small quantities of alkali ion can affect the titration. It has been the experience of chemists other than the Associate Referee that dilute silicofluoride solutions "do not titrate like the simple fluorides." The silica constituent very possibly may add a further complication.

Standardization of thorium nitrate solutions against the distillates from known amounts of pure fluoride may be a practical way out of the difficulty, but it does not solve the basic troubles involved.

The back-titration procedure developed by Dahle, Bonnar, and Wichmann (4), has both practical and theoretical advantages; by eliminating the distillate evaporation step it avoids complications resulting from the neutralization and evaporation of distillates (5) and saves the time involved in this process. It is, of course, possible to handle the total volume of a distillate when small quantities of fluorine are determined by the neutralization and evaporation process, but the back-titration procedure involves little loss of sensitivity in such cases if 100 ml. tall-form Nessler tubes, which permit the handling of aliquot volumes up to 90 ml., are used.

This modification of the thorium nitrate titration has passed through several stages since it was proposed. These are discussed in the order of their proposal, as follows:

A.—The original procedure (4) called for transfer of a distillate aliquot to a Nessler tube, and after the addition of 1.00 ml. of a 0.01 per cent solution of alizarin red, neutralization with 0.05 *N* sodium hydroxide to the transition point of the indicator. A "blank" tube with the same amount of indicator in a volume of distilled water corresponding to the aliquot taken was also provided. Then 2.00 ml. of 0.05 *N* hydrochloric acid was added to each tube (resultant *pH* at mark = about 2.75), and to the sample tube was added a dilute thorium nitrate solution to the end point of the indicator. This same volume of thorium solution was then added to the comparison tube, and the more highly developed resultant lake was bleached to an exact color match with the sample tube (when both were diluted to volume) by adding a measured volume of standard sodium fluoride solution. The fluorine content of the sample tube was considered to be equal to the quantity added to the blank tube in producing a match. The advantage of having a ready reference end point, which could be duplicated with delicacy under the same conditions, is apparent. However, it soon became evident that this procedure tended to yield slightly high results (6). This error was ascribed to the effect of alkali ion, introduced during the distillate neutralization, which prevented full development of the thorium-alizarin lake. Hence, slightly more than the theoretical amount of standard sodium fluoride solution was required to bleach to a match the fully developed lake in the comparison tube.

B.—In the last reference cited (6), a modification designed to eliminate this source of error is suggested. The acidity of the distillate was determined by titration of a suitable aliquot with 0.05 *N* sodium hydroxide. Another aliquot was placed in a Nessler tube for the fluorine titration, and its total acidity was adjusted to the equivalent of 2.00 ml. of 0.05 *N* acid in the 50 ml. tube by adding the proper amount of normal hydrochloric acid (or perchloric acid). The titration was then conducted as before. The details have been published (2), and a further refinement has been the use of a little hydroxylamine hydrochloride (7) in both tubes to discharge possible traces of chlorine liberated during the distillation due to slight decomposition of perchloric acid. (The quantity of hydroxylamine used was later increased.) With this modification of the back titration procedure it is noted that no alkali ion whatever is present in the sample titration tube, whereas  $\text{Na}^+$  ion is introduced into the blank tube when the back-titration with standard sodium fluoride solution is applied. The above statements indicate that the sodium ion, introduced with the standard fluoride in the titration, might possibly have an independent effect in coagulating or bleaching the thorium lake. This would lead to slightly low results. Such an effect was in fact found (7).

C.—An attempted refinement in this "acid substitution" procedure, or titration without distillate neutralization, involved a rough standardization of the thorium solution used against the standard fluoride solution. Then graphical relationship between the two solutions indicated approximately how much fluorine was present in the sample or "unknown" tube, and slightly less than this amount as standard sodium fluoride solution was mixed into the comparison or "blank" tube before the addition of any thorium solution. After the addition of the same amount of thorium solution as was added to the sample tube, only a slightly redder lake color was produced, and this was subsequently bleached to an exact match with additional small amounts of the standard sodium fluoride solution. It was intended by this modification to develop the lake in each tube in an approximately similar manner and thus to increase the accuracy of the titration. Such a procedure was used in obtaining the results on Solution A, described previously, where no distillation and resultant "acid compensation" were involved. However, and as noted, when the modification was applied to the *distillates* from fluoride solutions, the low results for Solution B were obtained. Apparently the modified procedure still fails to take into account the effect of alkali ion added along with the standard fluoride, and the low results on distillates are logically ascribed to the same source of error as appears in modification B.

Thus, neither modification B nor C allows for identical conditions of fluoride combination and salt concentration in both tubes, and these factors seem to be prerequisites for accurate results. The good results on Solution A can be reconciled with the low results on distillates from other

samples when these differences in the titration of a pure solution and of a distillate are considered.

*D.*—Thus, a further modification based upon the following reasoning was attempted. Knowing that fluorine distills as fluosilicic acid, the Associate Referee decided to neutralize the distillates before titration to approximately the  $pH$  of very dilute potassium fluosilicate solutions with standard potassium hydroxide and thus, presumably, to convert the evolved fluoride to potassium fluosilicate. The indicator used was a dilute alcoholic solution of *p*-nitrophenol, which becomes noticeably yellow at rather low  $pH$  (about 5.5) and is colorless at the  $pH$  of the back-titration (2.75). (As determined by the glass electrode, the  $pH$  of pure solutions of potassium fluosilicate were as follows for various concentrations of fluorine: 0.5 mg./ml. = 3.40; 0.01 mg./ml. = 3.77; 0.005 mg./ml. = 4.00.) Excess distilling acid (perchloric) is also neutralized at the same time, and in fact much the greater part of the alkali used in the neutralization is consumed by these traces of acid when small quantities of fluorine are distilled. Because distillates that had been allowed to stand for a time before neutralization (2 hours to overnight) appeared to show a diminishing fluorine titer, the practice was adopted of adding 1–2 drops of the *p*-nitrophenol indicator to the receiving flask at the beginning of the distillation and maintaining the distillate alkaline (faintest perceptible yellow) by the continued measured addition of 0.05 *N* potassium hydroxide at such a rate that the distillate was made alkaline, within one drop of alkali, as it came to mark. An aliquot of the neutralized distillate was selected for the back-titration as usual, and in order to compensate reasonably well for the amount of alkali ion added in the distillate neutralization, a corresponding amount of standard potassium salt (KCl or  $KClO_4$ , which, as the disturbing influence is the  $K^+$  ion, are interchangeable for the purpose) was added to the blank or comparison tube. The acidity was then adjusted by the addition to both tubes of the proper amount of 0.05 *N* hydrochloric acid, and the titration was conducted with a standard solution of pure potassium fluosilicate (instead of NaF) as the reference solution. The modification outlined in *C*, above, in which the greater part of the fluoride solution needed in the back-titration was added to the comparison tube before the addition of thorium solution, was retained.

As has been noted, results for both Solution B and the wine sample mentioned in the Associate Referee's last report (1), were low, and it was decided to repeat this work and to use modification *D* of the titration. It was hoped to ascribe the low results to improper titration technic rather than to losses in the distillation. Accordingly, corresponding samples were sent to collaborators for further study: one sample was a pure potassium fluosilicate solution containing 15.0 mg./liter of fluorine (Solution C), to which had been added sulfate and phosphate interference; the other



sample was a wine (0.2 p.p.m. "natural" or blank fluoride), to which had been added 7.0 p.p.m. of fluoride (weight/volume) as potassium fluosilicate. Analysis of both samples involved a distillation procedure, and analysis of the wine sample involved, in addition, the application of the ashing technic, with lime fixation, before the distillation and titration. The wine sample was of the high-sugar type, which has been known to cause problems of fluoride retention when the ashing procedure has been applied (7). Collaborators were asked to report on a weight per volume basis, and results are given in the first two columns of Table 1.

TABLE 1.—Average results of collaborators on fluoride

ANALYST*	SOLUTION C	WINE	"STRIP" 1	"STRIP" 2
	p.p.m.	p.p.m.	gr./lb.	gr./lb.
J. L. Hogan <sup>1</sup>	14.6	8.0	.018	.039
H. M. Risley <sup>2</sup>	13.5	7.2	.019	.041
H. M. Bollinger <sup>3</sup>	14.1	6.9	.017	.038
A. W. Hanson <sup>4</sup>	14.9	6.4	.016	.039
S. H. Perlmutter <sup>4</sup>	14.9	6.5	.015	.040
J. B. Snider <sup>4</sup>	14.7	6.5	.016	.041
C. R. Joiner <sup>5</sup>	14.8	7.0	.018	.040
P. A. Clifford <sup>6</sup>	14.9	7.1	.017	.040
General average	14.55	6.95	.0170	.0398
Contained	15.0	7.2	.018	.042
Per cent recovery	97.0	96.5	94.4	94.8

\* Address—U. S. Food and Drug Administration, at: <sup>1</sup>New York; <sup>2</sup>Seattle; <sup>3</sup>Los Angeles; <sup>4</sup>Minneapolis; <sup>5</sup>St. Louis; <sup>6</sup>Washington, D.C.

Average results on both Solution C and the wine sample are considerably better than those obtained last year on similar samples, and modification of the titration procedure is believed responsible for this improvement. Work by the Associate Referee on the distillation and titration of pure fluoride salts also yielded better recoveries with this technic than had heretofore been obtained.

The details of the revised titration procedure and also those of the sample preparation and distillation technic used in obtaining these and previous collaborative results have been published as a proposed general tentative method for fluoride (8).

The use of potassium fluosilicate as a standard in the titration made necessary the preparation of the pure compound. It was found that the salt as usually purchased contained a large proportion of potassium fluoride. Purification by recrystallization was impracticable because of low solubility, but it was found that a pure product could be obtained by the steam distillation of fluosilicic acid from an acid mixture of

fluoride and silica, and the collection of the distillate in potassium chloride solution. The precipitated potassium fluosilicate was washed free of chloride by centrifuging down with repeated portions of water, collected, and dried (8). The purity of the product has been determined both by means of a Travers' titration (9) and by a gravimetric conversion to potassium sulfate. An entirely pure product was not obtained with a glass condenser, as the evolved fluosilicic acid etched the glass noticeably and carried traces of silicate into the receiver; however, by the use of a platinum condenser a product yielding the theoretical titer by the Travers' titration and a 99.80 per cent figure for purity by the sulfate conversion was secured. (A portion of this product was furnished collaborators for the preparation of the standard solutions used in the present study.) Hence, while a good product can be obtained from glass, it is advisable not to accept it as gravimetrically pure, but to correct the weight taken in the preparation of standard solutions by a factor derived from the average of the above two methods of assay. G. L. Keenan (10) points out how silica, being doubly refracting and crystallizing in the trigonal system with significant refractive indices of  $n_e = 1.553$  and  $n_w = 1.544$ , is easily detected in the silicofluoride, which is singly refracting, crystallizing in the isometric system with  $n = 1.339$ . The purest product the Associate Referee was able to obtain, even with use of a platinum condenser, revealed a slight silica impurity, which could hardly be detected by ordinary analytical methods.

The general technic of the fluosilicic acid evolution of fluoride by means of an acid distillation has not been appreciably modified since it was originally proposed by Willard and Winter. Various forms of distilling apparatus have been proposed from time to time, but it is doubted if they have any significant advantage over the simple Claissen still employed by the original authors. However, use of all-glass apparatus of the Claissen type eliminates the need for rubber stoppers, an advantage when perchloric acid is employed as the distilling acid, and use of all-glass systems usually results in a lower "distillation blank (7)." Elaborate still traps are to be avoided in ordinary work because, while they aid in securing a low-acid distillate, their use promotes excessive refluxing and inefficiency in the distillation.

In certain cases, however, the use of an efficient trap may be helpful. In the Associate Referee's last report (1), it was mentioned how one investigator, W. E. Stokes, had privately stated that efficient trapping of the still eliminated traces of phosphoric acid in the distillate and thus made possible a fluorine determination on samples of phosphoric acid and food phosphate with only one distillation. Heretofore, a double distillation with intervening neutralization and evaporation of first distillates had been employed. As it was recognized that such a "short-cut" had considerable merit, investigations with low fluorine phosphoric acid and phos-

phates were conducted with a special still built according to the description given by Stokes. This was an all-glass Claissen type with the upright portion of the side-arm fitted with a bulb containing several dozen Pyrex beads. The inlet and outlet tubes of this trap were further bent in the manner of a Kjeldahl distillation bulb. In order to provide close control of refluxing, as well as of distillation temperatures, this bulb was enclosed in a jacket which could be held at any desired temperature, indicated by an inner thermometer, by means of a stream of air from the bench jet, heated by passing through a glass tube with an inner coil of resistance wiring controlled by a variable transformer. Before distillation the beads in the inner or scrubber bulb were always wet with a little of the distilling acid to supply a liquid acid phase.

It was found that 20.0 ml. portions of sirupy phosphoric acid could be distilled at 135°C. without the appearance of phosphoric acid in the distillate provided the scrubber jacket was unheated. If the scrubber was maintained at 125° (with the boiling mixture in the still at 135°), small quantities of phosphoric acid were distilled over, and progressively larger quantities appeared at higher reflux temperatures. It was determined that as small a quantity of phosphate ( $P_2O_5$ ) as 25 micrograms interfered in the titration of fluorine, giving results about 2.0 micrograms high when 25.0 micrograms of fluorine was titrated in the presence of various quantities of this interference. It was likewise found that distillates could be easily tested for the presence of interfering phosphoric acid by adding 5 ml. of a 1+9 dilution of Schricker's reagent (11) to 45 ml. of distillate in a 50 ml. cylinder, immersing in the steam bath for 10-15 minutes, and examining for the appearance of a blue or blue-green color (comparing against a blank) by looking down the length of the cylinder. Distilled fluoride in the quantities apt to be encountered in food work would hardly affect the delicacy of this test (12).

Because it was found to be impracticable to heat the scrubber, and because use of the unheated scrubber, coupled with the poor relative efficacy of phosphoric as a distilling acid, caused slow evolution of fluorine, it was necessary to collect much larger volumes of distillate than the usual 150-200 ml. (upwards of 500 ml. at 135°) before quantitative recoveries were approached. Similar observations were made when calcium phosphate samples were dissolved in and distilled from an excess of perchloric acid. However, the advantage of a single distillation, which eliminates a great part of the time and labor involved in a double distillation, is still apparent.

To test the practicability of the single distillation with phosphatic materials, two samples were sent to W. E. Stokes for assay by this technic. One was a sample of reagent phosphoric acid which had been steam distilled at 135°C. until fluorine free, then treated with pure sodium fluoride solution equivalent to 12.5 micrograms/ml.; the other was a portion of

the dicalcium phosphate used as a collaborative sample the previous year (1) and in the analysis of which the laboratory of W. E. Stokes did not participate. Results by R. L. Danielson with the single distillation technic on these samples were 11.6 micrograms/ml. of fluorine on the acid sample and 12.8 p.p.m. on the phosphate. In the latter case the true fluorine content was unknown, but the average of 12 former collaborators was 11.9 p.p.m. by a double distillation procedure. The higher result might well be the more nearly correct one. (The Associate Referee's figure on the phosphoric acid was 11.4 p.p.m. when 300 ml. of distillate was collected.) Twenty milliliters of the sirupy acid was taken for the analyses and distilled directly at 135°, and 10 grams of the phosphate with 20 ml. of perchloric acid was taken for the other sample. The volume of distillate collected in both cases was 400 ml., and tests with the Schricker reagent were conducted on 40 ml. portions of distillate to check for the presence of interfering phosphate. Danielson reported a trace of phosphate (5 micrograms  $P_2O_5$ ) in 40 ml. of the distillate from one 20 ml. sample of the phosphoric acid. This is hardly enough to interfere, and none was found in other distillates. Titrations were conducted with a standard solution of potassium fluosilicate according to modification *D* of the titration procedure. The still used by this collaborator was described as adapted from a 125 ml. Claissen flask with a simplified scrubber consisting of 12-13 grams of 6 mm. hollow glass beads, supported by several Vigreux indentations, built into the side-arm. The bead column was capped by placing over it the bottom  $\frac{3}{4}$  inch of a 15 mm. test tube, and the upper part of the side-arm was sealed off immediately above the outlet tube leading to the condenser.

Such results demonstrate the practicability of a fluorine assay upon phosphatic materials with one distillation provided special equipment is used, larger volumes of distillate (at least 400 ml.) are collected, and tests for the presence of interfering phosphate are made. However, the use of the glass bead scrubber is contraindicated, in ordinary work, because of the reason indicated. With the special jacketed apparatus built by the Associate Referee, only about 88 per cent recoveries were obtained in 150 ml. of distillate, with an unheated scrubber, when known quantities of fluorine were distilled from perchloric acid at 135°C. However, close to theoretical recoveries were obtained in only 100 ml. of distillate when the scrubber temperature was held at 125°, and distillate acidities still remained quite low. This would represent some saving in time, but it is doubtful whether the complications introduced by the use of such an apparatus are worth while. Good results are obtained from the simple Claissen still; however, a wise precaution, observed in obtaining the collaborative figures given in Table 1, is the sealing off of the side limb of the Claissen flask immediately above the outlet tube to prevent pocketing and excess refluxing of distillate. The simpler stills, in the Associate

Referee's experience, are the most efficient, and very good results have been obtained with ordinary 100 ml. Pyrex or Vycor distillation flasks when known quantities of fluorine were distilled. (No advantage resulting from the use of Vycor was noted, either in still efficiency or size of the distillation blank.) However, more adequate trapping than is provided by a simple distillation-type flask is usually necessary to prevent spraying over of distilling acid, especially when carbonate-containing ash samples are acidified in the still before the distillation. The still should employ a clean straight-tube condenser, no longer than is necessary for adequate cooling.

The precaution of cleaning the still with hot 10 per cent sodium hydroxide between runs is retained. This is especially important when siliceous samples have been distilled. In addition, it has been found that fuming out of a still with boiling sulfuric acid before runs appears to promote better recoveries and to minimize and render constant the distillation blank. This acid treatment may be necessary only occasionally if the still is in constant use, but it is helpful if it has not been used for some time. The use of a Wood's metal bath in conjunction with adequate shielding of the flask (7) is effective in the securing of a low acid distillate. A possibility, however remote, is that a flask may crack during a distillation and release the hot perchloric acid into the molten bath. For this reason flasks should be closely examined for cracks, and care must be taken that the metal is not too hot before a cold flask is immersed in the bath.

The use of a lime fixative is retained in the general ashing method of sample preparation. Details of this method and precautions to be observed in special cases during the sample preparation are given in the published part of this report (8).

A method for the analysis of fluoride spray residues on fresh fruits was mentioned in the last report (1). Subsequent collaborative trial of the method appears to prove its merit as a routine spray residue procedure, and experimental details are also given in the published part of this report along with the general fluorine method (8). Results of collaborators are listed in Table 2. The samples analyzed were portions of the hydrochloric acid-acidified and filtered "strip solutions" (13) of unsprayed apples to which had been added the indicated quantities of fluorine as gr./lb. on a 1400 gram sample weight basis plus contaminants as indicated. The fluorine, as pure sodium fluoride solution, and contaminants were added to the alkaline strip solution before acidification and filtration.

Recoveries are noted to be close to theoretical, tending slightly low. Further collaborative results on two more samples (undertaken a year later after very slight revision of the procedure) are given in the last two columns of Table 1. Added fluorine was on the same basis as that of a 1400 gram sample, and as the apples used were excessively dirty and heavily "stripped" no extra interferences were added. Results tend some-

TABLE 2.—*Results of collaborators—spray residue method*

Sample No.		1	2	3	4
F added (gr./lb.)		.008	.048	.035	.022
Contaminants (mg./ 500 ml. alkaline "strip solution")	$\left\{ \begin{array}{l} \text{SO}_4 \\ \text{P}_2\text{O}_5 \\ \text{Fe} \\ \text{Al} \end{array} \right.$	$\left\{ \begin{array}{l} \text{none} \\ 100 \\ 25 \\ 10 \end{array} \right.$	$\left\{ \begin{array}{l} 200 \\ \text{none} \\ \text{none} \\ 5 \end{array} \right.$	$\left\{ \begin{array}{l} 100 \\ 100 \\ \text{none} \\ 50 \end{array} \right.$	$\left\{ \begin{array}{l} 100 \\ 200 \\ 5 \\ \text{none} \end{array} \right.$
Average results of collaborators (gr./lb.)	A. K. Klein <sup>1</sup>	.0055	.0475	.0320	.0215
	A. J. Cox <sup>2</sup>	.0073	.0493	.0330	.0247
	P. A. Mills <sup>3</sup>	.0080	.0505	.0305	.0195
	H. M. Bollinger <sup>3</sup>	.0038	.0496	.0371	.0215
	J. F. Weeks, Jr. <sup>4</sup>	.0075	.0430	.0330	.0230
	J. L. Hogan <sup>5</sup>	.0096	.0500	.0365	.0206
	P. A. Clifford <sup>1</sup>	.0067	.0467	.0329	.0213
Average results (gr./lb.)		.0069	.0481	.0336	.0217
Per cent recovery		86	100	96	99

Address—U. S. Food and Drug Administration, at: <sup>1</sup>Washington, D. C.; <sup>2</sup>Seattle; <sup>3</sup>Los Angeles; <sup>4</sup>New Orleans; <sup>5</sup>New York; <sup>6</sup>Bureau of Chemistry, State Department of Agriculture, Sacramento.

what low, but the method, on account of flexibility and speed, appears adequate for a rapid assay of fresh fruit for spray residue fluorine and is accordingly proposed as a tentative procedure. Its principle has been successfully applied, with only slight modification of experimental details, to the analysis of fresh leafy vegetables for residue fluorine.

Thus, two methods have been presented for the consideration of the Association, a general procedure and another designed specifically for the spray residue determination (8). The general plan has been to establish methods similar in scope to the ones for lead, but not to exclude further work on other methods or their modifications, which may later be found superior from the standpoint of accuracy and simplicity. An effort has also been made to correlate and condense much scattered information into a connected method for fluorine that will be applicable to most food products. The efforts of many investigators are represented. This arrangement may be at some expense of space, but it will be to the advantage of the analyst in following the procedure.

#### REFERENCES

- (1) *This Journal*, 25, 394 (1942).
- (2) *Ibid.*, 23, 303 (1940).
- (3) *Ind. Eng. Chem., Anal. Ed.*, 5, 7 (1933).
- (4) *This Journal*, 21, 459 (1938).
- (5) *Ind. Eng. Chem., Anal. Ed.*, 11, 171 (1939).
- (6) *This Journal*, 21, 468 (1938).

- (7) *Ibid.*, 24, 350 (1941).
- (8) *Ibid.*, 27, 90 (1944).
- (9) *Ibid.*, 14, 253 (1931); *Methods of Analysis*, A.O.A.C., 1940, 50, 20.
- (10) Food & Drug Adm., Washington, D. C. (private communication).
- (11) *This Journal*, 22, 167 (1939).
- (12) *Ind. Eng. Chem., Anal. Ed.*, 14, 855 (1942).
- (13) *Methods of Analysis*, A.O.A.C., 1940, 407, 30.

## REPORT ON FOOD PRESERVATIVES AND ARTIFICIAL SWEETENERS

By W. F. REINDOLLAR (State of Maryland Department of Health,  
Baltimore, Md.), *Referee*

### *Benzoates and Esters of Benzoic Acid*

*Associate Referee*.—E. W. Boyce, Massachusetts State Health Department, Westfield, Mass.

*Recommendations of Subcommittee C, 1941*.—"(1) That studies be conducted on methods for the determination of benzoate of soda in foods.

"(2) That methods for the determination of the esters of benzoic acid be studied."

*Report*.—Owing to circumstances existing in his laboratory the Associate Referee was unable to start the problem until August 1943. He experimented with the Nicholl's test for the determination of less than 5 mg. of benzoic acid, which is based on the assumption that under standard conditions 12 per cent of the benzoic acid present is converted to salicylic acid, which is then determined colorimetrically against a standard solution of salicylic acid by means of the ferric chloride reaction. He believes this test shows promise, but suggests one or two modifications, the chief one of which is the preparation of standards by oxidizing known amounts of pure benzoic acid rather than employing salicylic acid. This would insure that any unknown factor or deviation that might occur would influence both sample and standards to an equal extent.

No work was done on the esters of benzoic acid.

The Referee recommends that the subject be continued.\*

### *Saccharin*

*Associate Referee*.—William F. Reindollar.

*Recommendations of Subcommittee C, 1941*.—"That studies be continued on methods for the detection and determination of saccharin."

*Report*.—The Associate Referee requested two laboratories to make preliminary determinations of saccharin in ginger ale by the proposed method (*This Journal*, 25, 369), in order to become familiar with the technic and

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 27, 62 (1944).

the apparatus employed. Neither laboratory was able to comply with this request, one because of the lack of a sublimator, the other owing to a shortage of personnel. The only work the Associate Referee has done has been to employ the method on several official samples.

The Referee recommends that the proposed method for the determination of saccharin be submitted to collaborative study.

### *Sulfur Dioxide*

*Associate Referee.*—C. E. Hynds, State Department of Agriculture and Markets, Albany, N. Y.

*Recommendations of Subcommittee C, 1941.*—"That an associate referee be appointed to study methods for the determination of added sulfur dioxide compounds in comminuted meats, which methods will take into account the oxidation of such compounds to sulfates after their addition to the meat."

*Report.*—In 1942 the associate referee developed a method for the determination of sulfur dioxide in meat which was quite similar to the Monier-Williams method and which he believed gave higher results. The method did not, however, measure the sulfur dioxide which had been oxidized to sulfate. As this loss may be considerable, particularly after a holding period such as occurs when samples are being shipped to the laboratory, the Referee requested that search be made for a procedure that would include these losses. Ashing and "soaking-out" procedures have been tried to this end but, so far, have not proved successful.

The Referee recommends that this study be continued.

### *Monochloroacetic Acid*

*Associate Referee.*—J. B. Wilson, Food and Drug Administration, Federal Security Agency, Washington, D. C.

*Recommendations of Subcommittee C, 1941.*—"That studies of methods for monochloroacetic acid be inaugurated."

*Report.*—The associate referee developed a method for the determination of monochloroacetic acid in beverages, involving the extraction of the substance with ether in a continuous extractor, followed by the hydrolysis of the acid and the volumetric determination of the chloride formed in the hydrolysis. This procedure, together with studies of its application to a number of beverages, was published in *This Journal*, 25, 145. As the method appeared quite promising the Referee requested that it be submitted to collaborative study but to date has received no further report from the associate referee.

The Referee recommends that the method for the determination of monochloroacetic acid in beverages developed by the associate referee be submitted to collaborative study.

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## REPORT ON SPICES AND CONDIMENTS

By SAMUEL ALFEND (U. S. Food and Drug Administration,  
St. Louis, Mo.), *Referee*

*Vinegar*.—Associate Referee A. M. Henry has submitted a report of work performed by himself and O'Neill on a "permanganate oxidation number," which he recommends for adoption as tentative. The Referee believes the method should first be subjected to collaborative study. Henry recommends that the modified Lichthardt test described in his report last year be adopted as tentative. Although the method is not infallible, it is by far the best available, and is needed. The Referee concurs in Henry's recommendation, with the proviso that further work be performed to clear up the difficulty with alkaline caramels.

Henry recommends that the method for total phosphoric acid in vinegar be made official (final action) and that the method for "Color removed by fullers' earth" be dropped. The Referee concurs.

*Mayonnaise and Salad Dressing*.—Associate Referee Sam D. Fine submitted his own and collaborative results on a method for starch in mayonnaise that is based on Field's application of Sullivan's method to mustard products. His recoveries are similar to those obtained by Field. There is indication that some of the starch in salad dressing undergoes a change on standing so that after several months the material does not yield as much starch by this method as it did when first prepared. Sufficient work has been done to warrant adoption of the method as tentative.

The 1939 report of the Referee took cognizance of an error in the sugar method, resulting from an incorrect reference, and undertook to correct it (*This Journal*, 23, 586, recommendation 13). This correction was adopted and published (*Ibid.*, 87). However, when the 5th edition of *Methods of Analysis* was published, an incorrect reference had again crept in. The associate referee's report for 1940 (*This Journal*, 24, 696) gives the correct reference. The method as published in *Methods of Analysis* should be corrected accordingly.

*Volatile Oil in Spices*.—No report was submitted by the associate referee.

*Mustard Products*.—Associate Referee J. T. Field did not submit a report. Since he is now in the Army, the subject should be reassigned and work should be continued.

## RECOMMENDATIONS\*

It is recommended—

(1) That the method for determination of total phosphoric acid in vinegar (*This Journal*, 24, 83) be made official (final action).

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\* For report of Subcommittee C and action by the Association, see *This Journal*, 27, 62 (1944).

(2) That the modified Lichthardt method for the detection of caramel in vinegar (*Ibid.*, 26, 234) be adopted as tentative, and that studies on the method be continued and include further collaborative work.

(3) That the "permanganate oxidation number" described in the associate referee's report be studied collaboratively, and be tested by the associate referee on as many different types of distilled vinegar and acetic acid as possible.

(4) That the tentative method for the determination of color removed by fullers' earth (*Methods of Analysis*, A.O.A.C., 76, 1940, 481) be dropped.

(5) That the tentative method for preparation of samples of mayonnaise and salad dressing (*This Journal*, 24, 83) be further studied.

(6) That the tentative method for the determination of total fat in mayonnaise and salad dressing (*Methods of Analysis*, A.O.A.C., 1940, 476 52; *This Journal*, 24, 83) be further studied.

(7) That the method for determination of starch in salad dressing described in the associate referee's report be adopted as tentative, and that studies on the method be continued.

(8) That the tentative method for the determination of volatile oil in spices (*Methods of Analysis*, A.O.A.C., 1940, 469, 16) be continued.

(9) That the tentative method for determination of starch in prepared mustard (*This Journal*, 25, 97-98) be further studied in its application to mustard flour, and that collaborative work on both prepared mustard and mustard flour be undertaken.

(10) That the official method for the determination of salt in prepared mustard (*Methods of Analysis*, A.O.A.C., 474, 36) be dropped (final action).

(11) That the official (first action) method for the determination of salt in prepared mustard (*This Journal*, 24, 703; 25, 98) be made official (final action).

(12) That the official method for the determination of volatile oil in mustard seed (*Methods of Analysis*, A.O.A.C., 1940, 472, 25) be studied in regard to its application to different types of seeds and to other mustard products.

(13) That the tentative method for the direct determination of moisture in spices (*This Journal*, 24, 83) be further studied.

(14) That studies on direct titration methods for the determination of moisture in spices be continued.

(15) That the tentative method for the determination of ash in spices (*This Journal*, 24, 83-4) be further studied.

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## REPORT ON MAYONNAISE AND SALAD DRESSING

By SAM D. FINE (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

At the 1941 meeting of the Association it was recommended by the referee (*This Journal*, 25, 66) that studies be continued on methods for the determination of starch in salad dressing. To carry out this recommendation the following work was undertaken.

The first method attempted was that for the determination of starch in peanut butter (*Methods of Analysis*, A.O.A.C., 1940, 420, 12). Trouble was experienced in completely extracting the oil without incurring mechanical losses of starch from samples of mayonnaise and starch. Modification of the procedure to overcome this difficulty was made; it was then found that the separated starch was not completely soluble in the Rask acid. Recovery of added starch was only 61 per cent. No further work was done with this method.

Field (*This Journal*, 24, 700; *Ibid.*, 25, 705) adapted a method by Sullivan (*Ibid.*, 18, 621) for the estimation of starch in plants to the determination of starch in mustard and prepared mustard. The method, as modified by Field (*loc. cit.*), was tried on samples of mayonnaise plus starch. The first recovery experiments were promising, and certain modifications were made in the procedure which led to even better results. The method follows:

## STARCH IN MAYONNAISE

## REAGENTS

- (1) *Calcium chloride solution*.—30 grams per 100 ml. of solution, adjusted to approximately 0.01 *N* alkalinity.
- (2) *Alcohol*.—95%.
- (3) *Alcohol*.—70%.
- (4) *Alcohol-sodium hydroxide solution*.—70 ml. of 95% alcohol plus 25 ml. of 0.1 *N* NaOH.
- (5) *Ammonium sulfate solution*.—Saturated aqueous solution.
- (6) *Iodine potassium iodide solution*.—2 grams of I plus 6 grams of KI in 100 ml. of water.

## PROCEDURE

Determine the total acidity of the prepared sample as directed in *Methods of Analysis*, A.O.A.C., 1940, 476, 49. Place 4–5 grams of the prepared sample in a 500 ml. Erlenmeyer flask, add the calculated quantity of 0.1 *N* NaOH to neutralize the acid in the weight of sample taken; and add 100 ml. of the CaCl<sub>2</sub> solution. Stopper the flask and swirl gently until all large lumps of mayonnaise (or salad dressing) are broken. Add glass beads. Wet the inside of a reflux condenser and the stopper with water, allow to drain for 1 minute, then connect flask to condenser. Place an asbestos board with a hole in the center under the flask and boil gently for 30 minutes, swirling from time to time. (Slight frothing occurs in the first few minutes.) Leaving the condenser connected, cool the flask and contents to room temperature in a pan of ice water. Remove the flask, stopper it, and shake vigorously. Pour the contents into a centrifuge bottle<sup>1</sup> and centrifuge for about 10 minutes at 1,500 r.p.m. Pipet a 50 ml. aliquot into a centrifuge bottle containing 150 ml. of 95% alcohol,

<sup>1</sup> Wide-mouthed 250 ml. bottles are the most convenient type. By tilting the bottle after centrifuging, 50 ml. aliquot may be removed without including any of the oil-containing upper layer.

stopper (rubber),<sup>2</sup> and shake vigorously for approximately 5 minutes. Rinse particles adhering to stopper into bottle with 70% alcohol; use same rubber stopper throughout the following steps. Centrifuge at 1,500 r.p.m. for approximately 10 minutes. Decant the supernatant liquid through an asbestos pad in a Caldwell crucible, using suction; retain the precipitated starch in the centrifuge bottle, adding 70% alcohol and recentrifuging if necessary. Pour off the supernatant liquid as completely as possible without transferring the starch to the crucible; 70% alcohol may be used in rinsing the mouth of the centrifuge bottle, and the washings may be allowed to pass through the Caldwell crucible. Remove the asbestos pad and transfer it to the centrifuge bottle. Rinse particles adhering to the crucible into the bottle with water. Add water to about 100 ml., stopper, shake bottle vigorously to break up precipitate, then add an excess<sup>3</sup> of the I-KI solution (2-3 ml.) and 50 ml. of the  $(\text{NH}_4)_2\text{SO}_4$  solution. Stopper, and shake bottle vigorously. Rinse particles adhering to stopper into centrifuge bottle with water, and centrifuge until clear. Decant supernatant liquid with suction through an asbestos pad in a Caldwell crucible. Add 50 ml. of the alcohol-NaOH solution to the precipitate in the centrifuge bottle, stopper, and shake vigorously; wash stopper with 70% alcohol, centrifuge, and decant through the same pad as before. Repeat treatment with the alcohol-NaOH solution until practically all the blue color disappears (usually 2-3 treatments are sufficient). Without centrifuging, transfer contents of the centrifuge bottle to the Caldwell crucible, using 70% alcohol to rinse the centrifuge bottle. Aspirate until pad is dry, then transfer pad to a 500 ml. Kjeldahl flask. Add 10 ml. of HCl (sp. gr. 1.1029) to the centrifuge bottle, stopper, and shake vigorously until all starch particles adhering to the walls are washed free or are in solution. Transfer the HCl to Kjeldahl flask. Rinse centrifuge bottle and crucible with small portions of water, adding washings to contents of Kjeldahl flask, until the total volume reaches approximately 60 ml. Loosen particles adhering to the inside of the Caldwell crucible with the aid of a rubber policeman. Attach Kjeldahl flask to a reflux condenser, first adding glass beads to lessen bumping, place an asbestos board with a hole in the center under the flask, and boil for 1 hour. Cool, nearly neutralize with 1 + 1 NaOH (use methyl orange as indicator), and filter into a 200 ml. volumetric flask. Wash Kjeldahl flask and contents of filter paper with water until volume reaches 200 ml. Mix well and determine dextrose as directed in *Methods of Analysis, A.O.A.C.*, 1940, 500, 37. Dextrose  $\times 0.9$  = starch. Correct for added volume of 0.1 N NaOH used to neutralize acidity of mayonnaise. Report percentage of starch in sample.

Table 1 gives the results on the recovery of various starches alone by the method. All recoveries are based on the values found on direct acid hydrolysis of the starches.

TABLE 1.—*Recovery of starches alone*

STARCH	RECOVERY per cent
Potato (1)	98.8
Potato (2)	96.6, 97.1
Corn	95.6
Arrowroot	96.7
Tapioca	97.1

Sullivan (*loc. cit.*) has a table showing recovery experiments on potato starch alone by his procedure. The recoveries are 100 per cent. An attempt was made to determine where the loss occurs in the work described here.

<sup>2</sup> A tight-fitting stopper that extends only 3-4 mm. into the mouth of the centrifuge bottle is preferable.

<sup>3</sup> Excess present when supernatant liquid after centrifuging has straw to brown color. If supernatant liquid is colorless, more of the I-KI solution must be added.

The following possibilities occurred to the Associate Referee: (1) The starch used contains reducing substances which are determined as starch on direct acid hydrolysis but which are removed in the procedure, and hence are not available in the final dextrose determination; (2) the treatment with boiling calcium chloride solution causes some hydrolysis of the starch, with subsequent loss before the final dextrose determination. The first possibility was checked by washing a weighed sample of cornstarch with 150 ml. of 95 per cent alcohol plus 50 ml. of 30 per cent calcium chloride solution, followed by washing with 70 per cent alcohol and subsequent acid hydrolysis. Recovery was 98.5 per cent as compared to direct acid hydrolysis of the cornstarch. This would indicate that there are not any reducing substances present in the starch which are removed by washing. An attempt was made to determine the presence of reducing substances in the supernatant alcohol-calcium chloride solution from a determination, after the starch was removed by centrifugation and filtration; it was very difficult to remove all the calcium; and no reducing substances could be detected in the calcium-free solution obtained. That the loss occurs during the first step is shown by Table 2, which gives results on starches alone when the iodine precipitation step was omitted.

TABLE 2.—*Recovery of starches alone, omitting iodine precipitation step*

STARCH	RECOVERY per cent
Potato (1)	95.9
Corn	96.4
Arrowroot	95.9
Tapioca	96.7

Table 3 gives the results on mayonnaise plus various starches by the method.

TABLE 3.—*Recovery of starches from mayonnaise plus starches*

STARCH	RECOVERY per cent	
Potato (1)	95.7	95.2
Corn	93.9	93.1
Arrowroot	94.7	95.7
Tapioca	96.0	
Corn + Arrowroot	92.9	93.3

A sample of mayonnaise was prepared containing 3.85 per cent potato starch (calculated from weight of air-dry starch weighed and direct acid hydrolysis value for the starch). Analysis the day after preparation gave the results shown in Table 4. It was intended to submit samples of this

TABLE 4.—*Recovery of starch from mayonnaise containing 3.85% potato starch*

STARCH FOUND per cent	RECOVERY per cent
3.69	95.8
3.81	99.0

mayonnaise for collaborative study, but after one week's standing separation occurred.

A second sample of mayonnaise was prepared, containing 4.94 per cent potato starch. Table 5 gives collaborative results obtained on this sample.

TABLE 5.—*Recovery of starch from mayonnaise containing 4.94% potato starch (prepared May 4, 1943)*

ANALYST	DATE OF ANALYSIS	STARCH FOUND		RECOVERY	
		per cent		per cent	
S. D. Fine	5/11/43	4.83		97.8	
	6/8 /43	4.72	4.63	95.5	93.7
I. Schurman	5/15/43	4.71	4.63	95.3	93.7
F. J. McNall	5/18/43	4.68	4.57	94.7	92.5
F. M. Garfield	6/ 1/43	4.52	4.48	91.5	90.7
H. P. Bennett	6/ 5/43	4.61	4.59	93.3	92.9
			4.57		92.5
C. R. Joiner	6/24/43	4.54	4.50	91.9	91.1

### SUMMARY

The method of Sullivan, as modified by Field and the Associate Referee, gives promising results when applied to the determination of starch in mayonnaise and salad dressing. Recoveries on starch alone are about 97 per cent. Recoveries of starch from mayonnaise plus added starch vary from 93 to 96 per cent. Recoveries on mayonnaise containing a known amount of starch analyzed shortly after preparation vary from 96 to 99 per cent; analyzed from a month to two months after preparation, recoveries vary from 91 to 96 per cent.

## REPORT ON VINEGAR

### DETECTION OF GLACIAL ACETIC ACID IN VINEGAR

By R. E. O'NEILL and A. M. HENRY, *Associate Referee* (Food and Drug Administration, Federal Security Agency, Atlanta, Ga.)

Solutions of glacial acetic acid have been substituted for distilled vinegars, and a method of detecting such substitution is needed.

Detection of formic acid in a product made from or containing wood-distilled acetic acid serves to reveal this particular adulteration,<sup>1</sup> but this test is not effective in detecting adulteration by catalytic glacial acetic that contains very little or no formic acid.

Work has recently been done in England on the detection of adulteration of vinegars by means of various common reagents. Following the

<sup>1</sup> *Methods of Analysis, A.O.A.C.*, 1940, 482, 79.

lead of these English investigators,<sup>3,4,5</sup> the writers carried out experiments, using permanganate and ceric sulfate oxidation, iodine absorption, sulfite and hydrochlorite binding, and ester values. The permanganate and iodine methods immediately showed promise, and of these two the permanganate titration method for measuring the content of reducing substances proved more satisfactory in distinguishing true distilled vinegars from diluted acetic acid solutions.

Caramel and the coloring matter from charred oak containers have a considerable permanganate oxidation value. In an attempt to separate or eliminate the effects of such color, dialysis, charcoal adsorption, and distillation were tried. Dialysis was found to be impracticable, as it required excessive time and completion was not easily obtained. Activated charcoal gave variable results, apparently due to incomplete adsorption. Reproducible results were obtained by distillation. Both partial and constant volume steam distillation was used in the trials.

The idea of oxidation values as a means of distinguishing vinegars was first utilized by A. Schmidt.<sup>6</sup> He titrated 50 ml. of vinegar and adjusted to 3 per cent acetic acid in the presence of dilute sulfuric acid with 0.1 *N* potassium permanganate to a permanent red color. The end point is variable, cloudy, and wholly unsatisfactory. In 1938, Edwards and Nanji<sup>4</sup> suggested distillation and determination of the oxidation value as follows:

*Distillation of Sample.*—Mix 60 ml. of the sample with 15 ml. of water in a 400 ml. distilling flask, add a little pumice, and distil the mixture slowly until exactly 60 ml. of distillate is collected.

*Oxidation Value.*—Measure 25 ml. of the distillate into a glass-stoppered bottle of about 200 ml. capacity, add 10 ml. of  $H_2SO_4$  (1+3) followed by 10 ml. of 0.1 *N*  $KMnO_4$  solution accurately measured. Allow the mixture to remain at 18°C. for exactly 30 minutes; then add 5 ml. of 10% KI solution and titrate the liberated iodine with 0.02 *N*  $Na_2S_2O_3$ , adding starch solution as an indicator at the end.

Carry out a blank determination at the same time, using 25 ml. of distilled water in place of the vinegar distillate.

Let the volume of 0.02 *N* thiosulfate used in the blank titration be *A* ml. and that in the test *B* ml., then

$$\text{Oxidation value} = 8(A - B).$$

This method was used as a basis for further work because it eliminated end-point difficulties. However, it introduces other difficulties. It often stops the reaction short of completion, and the reaction with 0.1 *N* permanganate proceeds at a diminishing rate, greatly influenced by temperature, time, and changing concentrations. This necessitates the setting up of an empirical procedure over a set of highly critical conditions.

<sup>3</sup> *Analyst*, 60, 705 (1935).

<sup>4</sup> *Ibid.*, 62, 553 (1937).

<sup>5</sup> *Ibid.*, 63, 410-19 (1938).

<sup>6</sup> *Ibid.*, 65, 594 (1940).

<sup>6</sup> *Z. Unters. Lebensmittel.*, 69, 472 (1935).

TABLE 1.—*Effect of temperature fluctuations*

TEMP.	TITRATION 50 ML. UNDILUTED VINEGAR (+10 ML. DIL. H <sub>2</sub> SO <sub>4</sub> ) WITH <i>N</i> KMnO <sub>4</sub>	TITRATION 25 ML. VINEGAR+75 ML. H <sub>2</sub> O+10 ML. DIL. H <sub>2</sub> SO <sub>4</sub> WITH 0.1 <i>N</i> KMnO <sub>4</sub>	10 ML. VINEGAR+ 90 ML. H <sub>2</sub> O+10 ML. DIL. H <sub>2</sub> SO <sub>4</sub> WITH 0.1 <i>N</i> KMnO <sub>4</sub>	5 ML. VINEGAR +95 ML. H <sub>2</sub> O +10 ML. DIL. H <sub>2</sub> SO <sub>4</sub> WITH 0.1 <i>N</i> KMnO <sub>4</sub>
	TIME 1 HOUR ml.	TIME 1 HOUR ml.	TIME 1 HOUR ml.	TIME 1 HOUR ml.
10	6.70	9.0	3.6	1.6
20	7.35	13.5	6.5	3.2
25	7.55	14.1	7.3	3.7
30	7.80	15.0	7.9	4.2
35	8.00			
45	8.70			
60	9.10			
75	11.10			

Consequently it was deemed necessary to study the reaction closely with a view to discovering the optimum conditions for the method.

*Temperature.*—Experiments showed that temperature fluctuations cause relatively great changes in titration values, and consequently the temperature should be closely controlled; they also showed that normal room temperatures were somewhat less critical than the higher ones, which are more difficult to maintain. Therefore 25°C. was selected as the temperature at which the reaction is to be carried out. Table 1 gives the condensed results of the experiments.

Figure 1 shows these results graphically.

*Time.*—It was known that the rate of reaction was not rapid and that it diminished as concentrations changed. Experiments showed that the time factor and concentration, particularly of the permanganate, are interdependent, but that use of the stronger reagent for one hour carried the reaction onto a part of the time-titration curve which was least critical.

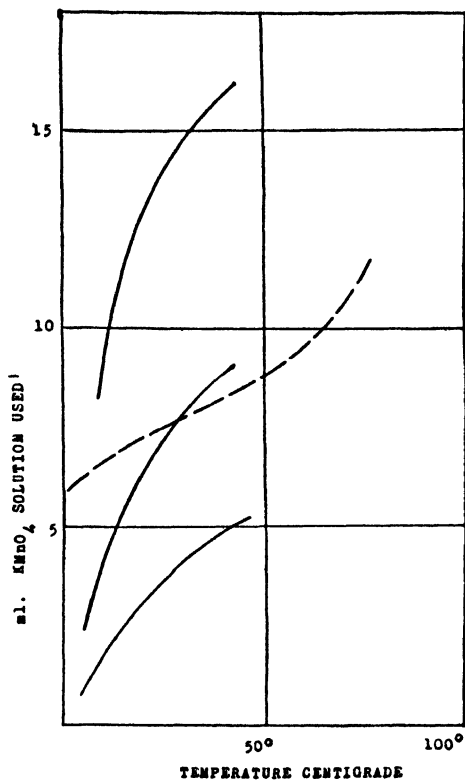


FIG. 1.—BROKEN LINE INDICATES USE OF NORMAL KMnO<sub>4</sub> WITH UNDILUTED VINEGAR. SOLID LINE INDICATES USE OF 0.1 KMnO<sub>4</sub> WITH VINEGAR DILUTED TO 25% IN THE TOP CURVE, TO 10% IN THE MIDDLE CURVE, AND TO 5% IN THE BOTTOM CURVE.



TABLE 2.—*Effect of time of reaction*

REACTION TIME	TITRATION OF 50 ML. UNDILUTED VINEGAR+ 10 ML. DIL. $H_2SO_4$ WITH Normal $KMnO_4$ AT $25^\circ C$ .	TITRATION OF 50 ML. VINEGAR +50 ML. $H_2O$ +10 ML. DIL. $H_2SO_4$ WITH 0.1 N $KMnO_4$ AT $25^\circ C$ .
Minutes		
5	6.50	
10		5.50
15	7.45	
20		8.15
30	7.85	10.35
40	7.90	12.00
45	8.00	
50		13.00
60	8.15	13.65
90	8.30	14.95
120	8.60	15.65
150		16.05
180	8.80	16.25
210		16.35
240	8.95	

Figure 2 illustrates graphically the meaning of the time-titration experiment.

*Concentrations.*—A comparison of the tenth normal and normal titration curves against temperature and time (Figures 1 and 2) illustrate the effect of varying the concentration of the potassium permanganate. It is seen that the optimum concentrations of reacting substances depend upon temperature, but especially upon time. Consequently it is difficult to discuss reacting concentrations without continual reference to the time factor. In fact, all three variables had to be worked out more or less concurrently.

As experimentation continued it became evident that use of the highest practicable concentrations of permanganate gave the best results.

TABLE 3.—*Effect of 0.1 N and normal  $KMnO_4$* 

VINEGAR USED ml.	0.1 N $KMnO_4$ REQUIRED ml.	NORMAL $KMnO_4$ REQUIRED ml.
0	.0	—
5	4.0	—
10	8.95	1.80
15	12.70	—
20	14.45	3.50
25	15.65	—
30	16.30	5.10
35	16.50	—
40	—	6.65
45	—	—
50	—	8.30

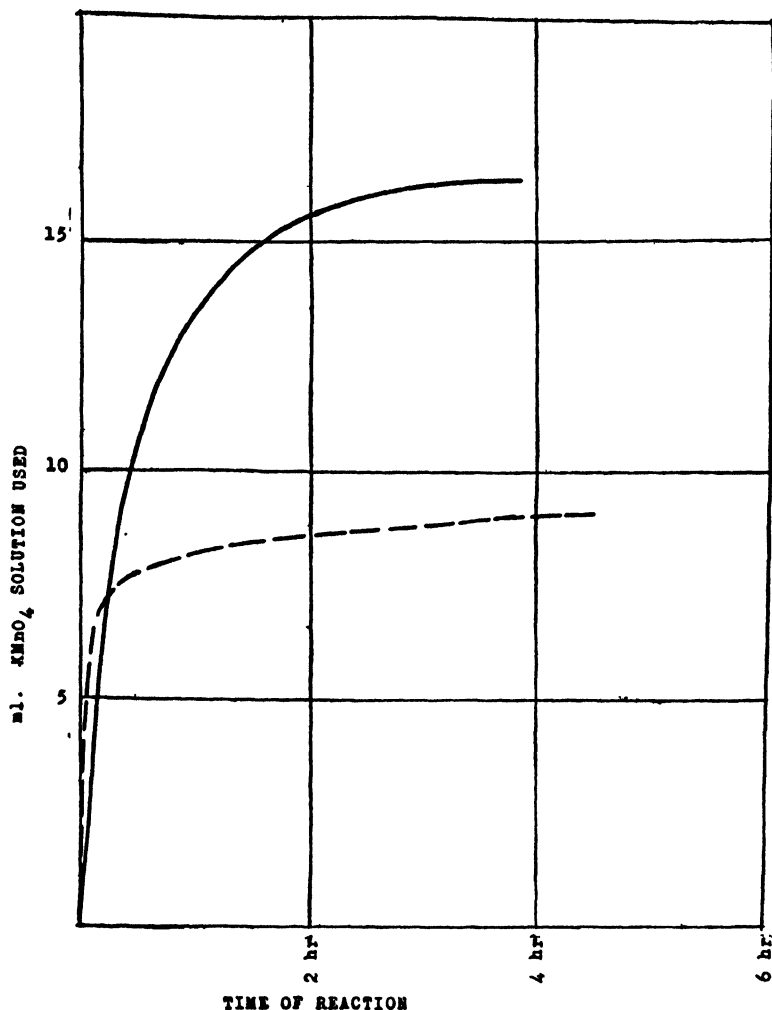


FIG. 2.—BROKEN LINE INDICATES USE OF NORMAL  $\text{KMnO}_4$  WITH UNDILUTED VINEGAR. SOLID LINE INDICATES USE OF 0.1 N  $\text{KMnO}_4$  WITH VINEGAR DILUTED TO 50%.

In all the above determinations the usual procedure was followed; it will be given in a statement of method later.

It is obvious from the figures shown that the reaction between permanganate and the organic reducing materials does not go to completion. In any such reaction concentrations have a marked effect, and if both reacting substances are permitted to become very dilute at the same time, as happens near the end of any direct titration, the rate of reaction diminishes almost to the stopping point before completion. It was this

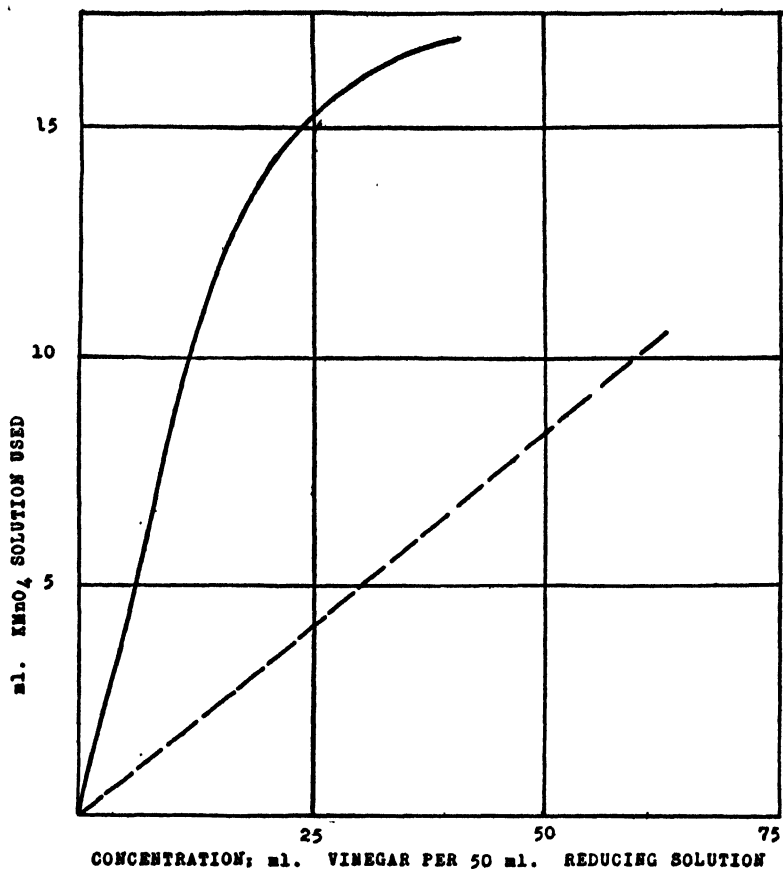


FIG. 3.—BROKEN LINE INDICATES USE OF NORMAL  $\text{KMnO}_4$ .  
SOLID LINE INDICATES USE OF 0.1 N  $\text{KMnO}_4$ .

condition that made the direct titration end-point of A. Schmidt very difficult to determine. The procedure of Edwards and Nanji, which adds an excess of the conventional tenth normal permanganate, reacts for a given time, and titrates the excess permanganate, showed the way to eliminate the difficulties.

These titrations are of course carried out in sulfuric acid solutions. In fact, experiments showed that an acid condition was necessary to get any titration at all. It was also shown that at ordinary temperatures an acid concentration of 3 normal would cause permanganate and acetic acid to begin to react. So a normal concentration of sulfuric acid was agreed upon. The addition of 10 ml. of 1 to 1 sulfuric acid in a total reaction mixture of 80–100 ml. would approximately accomplish this.

These experiments show that in order to obtain a condition in which a

uniform rate of reaction is maintained, the concentration of the permanganate in the reaction mixture must not drop below .15 normal when the titration is finished. The results given in Table 4 indicate that this is true.

TABLE 4.—*Effect of concentration of permanganate*

VINEGAR USED	NORMAL KMnO <sub>4</sub> ADDED	0.5 N H <sub>2</sub> SO <sub>4</sub> USED	TOTAL VOL.	NORMALITY OF KMnO <sub>4</sub> IN MIXTURE—		NORMAL KMnO <sub>4</sub> USED
				AT BEGINNING OF TITRATION	AT END OF TITRATION	
ml.	ml.	ml.	ml.			ml.
50	15	16.2	75	.20	.09	6.90
50	25	35.5	85	.29	.21	7.25
50	35	55.8	95	.37	.29	7.10
50	50	86.6	110	.45	.40	6.70
50	15	16.9	110	.14	.06	6.55
50	25	36.0	110	.23	.16	7.00
50	35	56.2	110	.32	.25	6.90
50	50	86.5	110	.45	.40	6.70

It is seen that the optimum condition, that is the largest permanganate titration, was obtained in the cases where 25 ml. of normal permanganate was added. The best results came when the solution was .25–.3 *N* with respect to permanganate to start with and did not fall below .15 at the end.

It is seen that the key factor is the concentration of potassium permanganate in the reaction mixtures. It must be kept above a certain minimum level to maintain such a rate of reaction that the permanganate used is proportional to the amount of reducing materials present, that is, the titration curve is a straight line. It is further seen that this condition obtains in all cases where the concentration of permanganate in the reaction mixture is maintained above .15 *N*. This means that some normality much greater than tenth normal for the permanganate must be used. Normal solutions are easily prepared at ordinary temperatures and with the usual precautions have proved satisfactorily stable.<sup>7</sup> No dilutions of the reaction mixture beyond what is absolutely necessary are permissible. The vinegar itself is adjusted to 4 per cent acetic acid instead of the 3 per cent of the Schmidt procedure. The sulfuric acid is diluted only 1 to 1 before using, just enough to remove the heat of mixture.

The results up to this point indicate that conditions should be fixed as follows: At least a 50 ml. sample of adjusted vinegar is necessary to obtain significant titrations. Convenience and the necessity for strong permanganate concentration make it desirable to use 25 ml. of normal permanganate. With 10 ml. of 1 to 1 sulfuric acid the concentration of permanganate in the reaction mixture to start is .29 *N*. If half the permanganate is used (12.5 ml.) the concentration at the end is .15 *N*—the

<sup>7</sup> *Methods of Analysis, A.O.A.C.*, 1940, 653.

lowest permissible value. Practically all the types of vinegars will be taken care of by a 12.5 ml. net titration with a good margin for safety. If the vinegars require more than a 12.5 ml. titration they can be diluted to such a fraction of their former concentration as will titrate less than 12.5 ml. and allowance can be made in the calculation of results.

**Distillation.**—The distillation part of the procedure presented no complications, and no difficulty was experienced in getting check between duplicate samples. The volatile and non-volatile substances appear to vary widely in boiling point so separation offers no obstacle. Any method of distillation can be made to serve but steam distillation appears to give the best results, and the standardized set-up of Clark and Hillig<sup>a</sup> for volatile acid work presents a convenient uniform procedure.

The details of the entire method follow:

#### METHOD

##### REAGENTS

(a) *Acetic acid solution.*—Glacial. To 4 grams of acetic acid add 100 ml. of water. This solution should have a negligible permanganate oxidation number when determined according to this method.

(b) *Sulfuric acid solution.*—Mix equal volumes of  $\text{H}_2\text{SO}_4$  and water.

(c) *Potassium iodide solution.*—Dissolve 30 grams of KI in 100 ml. of distilled water and filter. Do not use after discoloration with iodine.

##### APPARATUS

For the steam distillation use the standard Clark-Hillig volatile acid still. (As an alternative an all-glass apparatus is preferable.) If all-glass apparatus is not available, cover all cork or rubber stoppers with tin or aluminum foil.

##### DETERMINATION

Adjust the vinegar to 4 grams per 100 ml. acidity as acetic by dilution with distilled water. Steam distil 50 ml. of adjusted vinegar, maintaining the volume so as to have a residue of 45 ml. for 50 ml. of distillate. Keep distillate and reagents at 25°C. Transfer the 50 ml. distillate to a 250 ml. glass-stoppered Erlenmeyer flask and add 10 ml. of the 1:1  $\text{H}_2\text{SO}_4$  and 25 ml. of normal  $\text{KMnO}_4$  solution. Hold at 25°C., preferably in water bath for exactly 1 hour. Then immediately add 20 ml. of 30% KI solution and mix well. Titrate the freed iodine with 0.5 N  $\text{Na}_2\text{S}_2\text{O}_3$  solution. Run a blank of the acetic acid solution with the sample and subtract the ml. of normal permanganate solution used from the 25 ml. before subtracting the ml. of 0.5 N  $\text{Na}_2\text{S}_2\text{O}_3$ .

To obtain the permanganate oxidation number divide by 2 the ml. of 0.5 N  $\text{Na}_2\text{S}_2\text{O}_3$  and subtract this from 25 minus the blank. If the permanganate oxidation number is more than 15, repeat, taking half the original amount of vinegar. (It is necessary to repeat this reduction by half until the ml. of normal permanganate used is less than 15.) Then multiply the titration by the appropriate factor to obtain the permanganate oxidation number on the basis of 50 ml. of vinegar.

Additional information is acquired by obtaining the permanganate oxidation number of 50 ml. of the adjusted vinegar without distillation and also of the undistilled residue made up to 50 ml. with the 4% glacial acetic acid.

<sup>a</sup> *This Journal*, 21, 685 (1938).

Table 5 gives typical results with diluted glacial acetic acids and various vinegars. It is seen that on diluted glacial acetic acids, colored or uncolored, a permanganate oxidation number of less than 0.5 is obtained on the distillate. All distilled and other vinegars examined to date gave numbers of more than 3.0 on the distillate.

TABLE 5.—*Typical results with acids and vinegars*

	BEFORE DISTILLATION	DISTILLATE	UNDISTILLED RESIDUE
Destructively distilled glacial acetic acid diluted	0.0	0.1	0.0
Above, colored with caramel	0.8	0.1	0.8
Synthetic glacial acetic acid diluted	0.0	0.1	0.0
Above, colored with caramel	0.9	0.1	0.7
Above, colored by soaking with charred oak chips	13.6	0.3	12.6
Distilled vinegar (a)	7.9	6.2	1.0
Distilled vinegar (b)	5.1	3.4	0.9
Distilled vinegar (b) colored with caramel	6.0	3.3	1.9
Molasses vinegar	15. +	3.3	15. +
Cider vinegar	15. +	3.5	15. +

For recommendations on vinegars, see the report of the Referee on Spices and Condiments and also the report of Subcommittee C (*This Journal*, 27, 62 (1944)).

#### APPOINTMENTS

Sutton Redfern, Fleischmann Laboratories, New York City, has been appointed Associate Referee on Proteolytic Activity of Flour, in place of Quick Landis, deceased.

F. M. Strong, College of Agriculture, University of Wisconsin, has been appointed Associate Referee on Nicotinic Acid.

Merlin Mundell, Drug Division, Food and Drug Administration, Washington, D. C., has been appointed Associate Referee on Demerol.

Bert J. Vos, Jr., Division of Pharmacology, Food and Drug Administration, Washington, D. C., has been appointed Associate Referee on Digitalis Preparations.

W. H. King, State Department of Health, New Orleans, La., has been appointed Associate Referee on Chlorine in Milk.

H. W. Conroy, Food and Drug Administration, Kansas City, Mo., has been appointed Associate Referee on Ethylaminobenzoate in Ointments.

#### CORRECTIONS FOR VOLUME XXVII, NO. 1

Page 72 (14), line 2, transpose "36" and "54"; line 3, change "47" to "69."

Page 80, line 6, (1), change  $\frac{I_0}{I_0}$  to  $\frac{I_0}{I}$ .

Page 88, line 4, change minus sign to equality sign.

Page 89, line 3, change "39-41" to "78."

Page 98, 10, line 8, change "renewal" to "removal."

Page 111, (6), line 6, change "524" to "425."

## CONTRIBUTED PAPERS

### AN INNOVATION IN TECHNIC OF CITRATE DIGESTIONS\*†

By W. H. MACINTIRE, H. L. MARSHALL, and T. A. MEYER (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.)

The A.O.A.C. method for the chemical appraisal of commercial fertilizers stems from the procedure evolved by Frezenius, Neubauer, and Luck in 1871 (3). Their method was intended solely for the analysis of superphosphate and acidic types of "mixed goods." It predicated that the citrate digestion would be restricted to analytical residues devoid of water-soluble phosphates and substantially depleted of concomitant calcium sulfate and calcium fluoride. Neutral ammonium citrate was selected as the solvent for any water-insoluble phosphates generated during acidulation, curing, and analysis. A primary consideration in the selection was the inertness of the buffered citrate solution toward rock phosphate residues. For its intended utility the method was most satisfactory, notwithstanding the early difficulties encountered in the preparation of an exact citrate solution. Unfortunately, however, the analytical function of the citrate has been distorted and extended unduly. When used to measure solubility by the direct digestion of analytical charges of processed basic phosphates, the citrate is burdened with substantial proportions of salts similar to those that are removed in the prescribed washing of acidic phosphates.

Ammoniation is a relatively recent development (10), and therefore it was not contemplated that the Frezenius, Neubauer, Luck (3) procedure would be utilized in the analysis of ammoniated superphosphates. Moreover, the admixing of limestone, in other than small quantities for conditioning, was frowned upon until the use of dolomite was advocated (11, 12). Both of these processings effect a substantial percentage diminution of water-soluble phosphates and thus lessen their removal in the prescribed analytical step of aqueous extraction. The resultant concentration of calcium salts affects the *pH* and the dissolvent capacity of the citrate solution. There was, however, no alternative method for the analysis of the modern commercially-processed neutral and basic fertilizers. Recently a method was proposed for the direct determination of  $P_2O_5$  (16).

The vitiative effect of the additive bases upon analytical values for  $P_2O_5$ -availability has been recognized and subjected to critical and collaborative studies. These have dealt with the effect of the *pH* of the citrate solution; filtration technic; common-ion effect; ratio of charge to volume of the citrate; temperature and variation in agitation technic;

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\* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C. October 27, 28, 1943.

† The present study was conducted in the Cooperative Chemical Research Unit of the Department of Chemical Engineering, Tennessee Valley Authority, at the Agricultural Experiment Station of the University of Tennessee.

duration of the digestion and also washing with an electrolyte solution (1, 4, 5, 6, 7, 8, 9, 11, 13, 14, 15, 16, 17, 18, 19, 20). The referee studies resulted in the adoption of a 1-gram analytical charge and a 1-hour digestion instead of the 2-gram charge and 30-minute digestion (20). This modification induces higher "availability" values. Nevertheless, the associate referee concluded (20, p. 266) "The proposed method thus appears to be a decided improvement over the present official method as a means of fertilizer control, but neither method is adapted to give a fixed rating for the fertilizer efficiency of the water-insoluble phosphates." Recently, Andrews (2) pointed out that plant response to ammoniated phosphates is less than the expectancy indicated by the official method.

That procedure now prescribes that the interval between the completion of washing and the introduction of the water-insoluble residue into 100 ml. of neutral ammonium citrate of 1.09 sp. gr. be not longer than one hour. This provision precludes appreciable loss in  $P_2O_5$  availability through the fluoride reaction postulated by MacIntire, Hardin, Oldham, and Hammond (17), and demonstrated by Ross and associates (1, 4, 18) to be a factor when the leached charges are allowed to stand overnight precedent to the citrate digestion.

#### WATER BATH DIGESTER VERSUS DRY HEAT CHAMBER

So far as the writers have been able to ascertain, citrate digestions have been conducted only in water baths. To assure that the flask will be submerged to a depth equal to that of the contained citrate solution, the flasks may be weighted with a metal collar, or the bath may be supplied with a false bottom. Without agitation for the water content of the bath, some variance in spot temperatures is to be expected. This is shown for an electrically-heated water bath in Figure 1.† The spot temperatures of the contained citrate solvent in the twelve 100 ml. digestates also are shown. The flasks rested upon a perforated shelf about two inches below the surface of the 12-inch depth of water. The immersion depth, therefore, was slightly greater than the depth of the citrate content of the flasks. Initial heating of the bath was expedited by means of an auxiliary strip heater. The bulb of a calibrated thermometer was submerged at spot No. 11 to a mid-depth of the 12 inches of water. When 65°C. was attained, two thermometer readings were made also at each of the other eleven spots. These paired readings recorded temperatures of the water in immediate contact with opposite sides of the flasks at the shelf level. The same pair of thermometers was used to ascertain every spot temperature. The temperatures of the digestates were obtained simultaneously by means of calibrated Anschütz thermometers, the bulbs being submerged fully on center in the digestates, without touching the bottom of the flasks.

† The electrically-heated chamber was described and certain findings were presented under different title before the Fertilizer Section of The American Chemical Society at its 160th meeting, Pittsburgh, Pa., September, 1943. That paper was released for publication in *This Journal*.



Only at spot 12 was the temperature of 65°C. maintained in a laboratory having a temperature mean of 29°C. The lowest bath temperature registered was that of 63.5°C. at spot No. 2 and the mean of the water temperatures at the 12 points was 64.1°C. No digestate attained

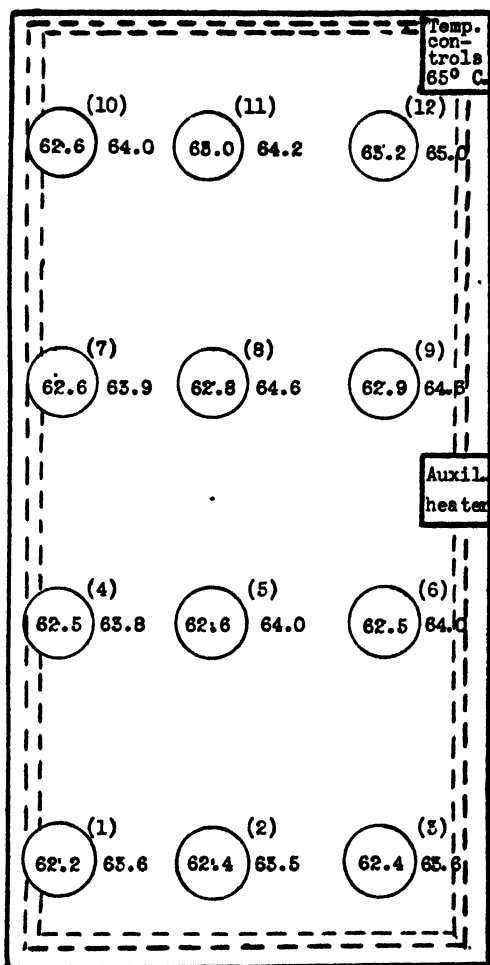


FIG. 1.—VARIANCE IN SPOT TEMPERATURES OF DIGESTATES AND SURROUNDING WATER IN A BATH HEATED BY A SUBMERGED ELECTRIC ELEMENT

65°C., and none reached the temperature registered by the 63.5°C. minimal of the water spots. The digestate temperatures varied in the range of 62.2°–63.2°C., with a mean of 62.6°C.

Obviously, the 12 agitations at 5-minute intervals monopolize the attention of the analyst during the 1-hour digestion. These factors and the

personal equation in manual agitation militate against reproducibility of analytical results. The present study was initiated to test the efficacy of constant agitation of the digestate in the dry air of a well-insulated elec-

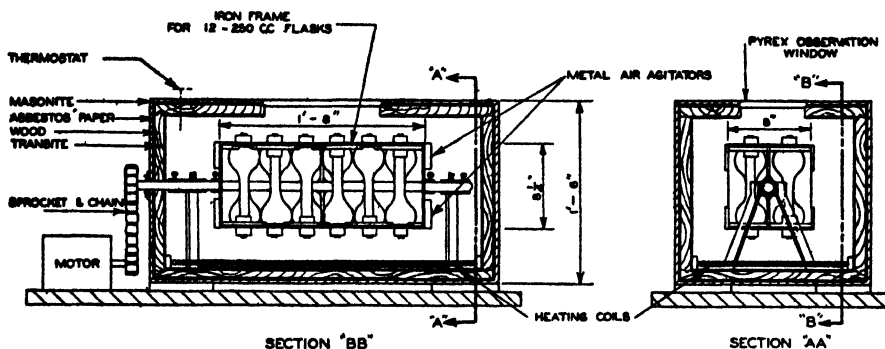


FIG. 2.—ELECTRIC AIR BATH FOR CONSTANT AGITATION OF CITRATE DIGESTIONS.

trically-heated chamber, wherein temperature is virtually the same at all points. The dry-heat chamber proposed for citrate digestion with constant agitation is diagramed in Figure 2, and the interior is shown in Plate 1.

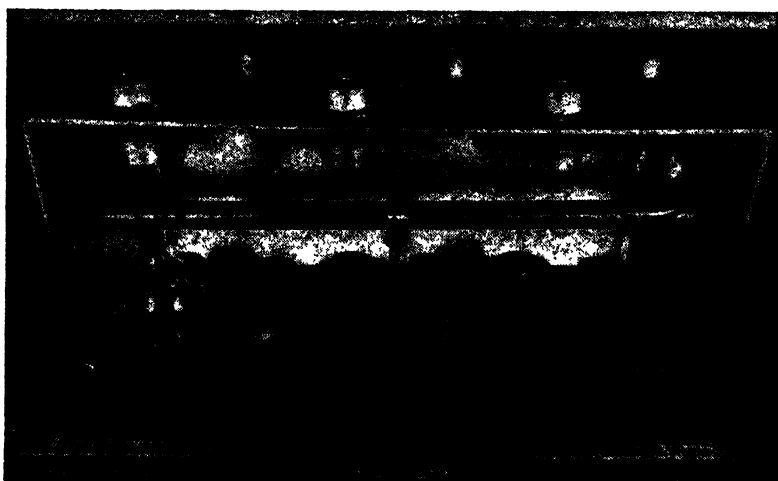


PLATE 1.—INTERIOR VIEW OF ELECTRIC AIR BATH FOR CONSTANT AGITATION OF CITRATE DIGESTIONS

It embodies features adopted after many tests with several heating units and air baffles. Heat is supplied by a single nichrome wire that extends along the four walls and constancy of temperature was assured by a

thermoregulator. A small bulb serves to illuminate the interior of the chamber, which can be inspected through a glass panel in the lid. Since the current consumption is low, the atmosphere of the chamber is maintained at 65°C. continuously to expedite initiation of digestions, unless an extended period of non-use is anticipated. The chamber accommodates twelve 250 ml. Erlenmeyers, which are revolved end-over-end at 20 r.p.m. With proper set-up the use of the electric heat chamber conserves the attention and time of the analyst and it is also cleaner and more economical than the water bath. Analytical work is facilitated and expedited by maintenance of a stock solution of citrate at 65° C. Starting without the pre-heated stock solution the solutions are heated-agitated in the ever-heated chamber prior to introduction of the residues.

#### ACCENTUATIVE EFFECT OF CONTINUOUS AGITATION IN CITRATE DIGESTIONS

The findings of Table 1 were obtained by use of referee samples of known history. It is evident that periodic agitation during the 1-hour digestion increased the dissolvement of the water-insoluble phosphates for those materials that contain appreciative percentages of citrate-insoluble  $P_2O_5$ . Still greater dissolvent action resulted from continuous end-over-end agitation in the electrically-heated chamber. The citrate-insoluble values obtained by the use of the heat chamber were as low or lower than those obtained by the present official technic. Moreover, reproducibility of analytical results was much better for the agitated digestates than for those obtained by periodic agitation.

#### INFLUENCE OF VARIATION IN TEMPERATURE DURING CONSTANT AGITATION

The nine phosphates (Table 1) were used also in comparisons to ascertain whether continuous agitation in the chamber would admit of digestion at a temperature below 65°C. The results given in Table 2 show that for elevation in temperatures of 30 to 40 to 50°C. there was a progressive decrease in percentages of C.I. Continuous agitation at those temperatures, however, did not compensate for a lowering of digestion temperature. In five cases continuous agitation and temperature of 60°C. was as effective as the official technic. Small variations were found for the highly-ammoniated superphosphate, but the outstanding exception was the anhydrous dicalcium phosphate. Hence, the 60°C. digestions with constant agitation would not be admissible without qualification, although they would be adequate for acidic materials and processed products having low content of generated basic phosphates. Again, however, every continuously-agitated digestate at 65°C. gave a C.I. value below the corresponding value obtained by means of the official procedure.

TABLE 1.—*Accentuative effect of continuous agitation on 1-hour citrate digestions at 65°C. in the determination of citrate-insoluble  $P_2O_5$  (per cent)*

A.O.A.C. REFERENCE SAMPLE				P <sub>2</sub> O <sub>5</sub> VALUES						
TYPE	NH <sub>4</sub> CONTENT	NUMBER	YEAR	TOTAL	WATER-SOLUBLE	CITRATE INSOLUBLE				
						A.O.A.C. BATH		(3) ELECTRIC OVEN; CONTINUOUS AGITATION	VARIANCE FROM (2)	
						(1) NO AGITATION	(2) PERIODIC AGITATION*		BY (1), NO AGITATION	BY (3), CONTINUOUS AGITATION
Superphosphate	—	A-1	1931	20.44	17.17	0.03	0.03	0.008	0.00	-0.022
Ammoniated superphosphate	2.48	A-10	1931	20.63	12.61	1.96	1.97	1.72	-0.02	-0.25
Ammoniated superphosphate	7.35	A-9	1931	18.79	1.53	6.70	6.35	6.15	+0.35	-0.20
Ammoniated superphosphate	8.66	A-13	1931	19.25	0.20	7.35	6.93	6.85	+0.42	-0.08
Dicalcium phosphate†	—	2	1936	51.75	0.15	4.65	2.60	1.98	+2.05	-0.62
Calcined phosphate	—	10	1936	36.88	0.05	4.95	3.65	3.48	+1.30	-0.17
Calcium metaphosphate	—	13	1936	61.88	5.65	0.19	0.08	0.06	+0.11	-0.02
Fused phosphate	—	14	1936	26.79	0.11	4.40	3.75	2.95	+0.65	-0.80
Mixed fertiliser (5-9-4)	4.00	6	1935	9.88	0.67	0.78	0.79	0.75	-0.01	-0.04
Number of plus values									6	
Mean of plus values									0.81	
Number of minus values									3	9
Mean of minus values									0.01	0.24

\* At 5-minute intervals.

† This product was not a true Ca HPO<sub>4</sub>.

TABLE 2.—Effect of variation in temperature of 1-hour citrate digestions under continuous agitation in the determination of citrate-insoluble  $P_2O_5$  (per cent)

A.O.A.C. REFERENCE SAMPLE				P <sub>2</sub> O <sub>5</sub> VALUES																	
TYPE	NH <sub>3</sub> CON- TENT	NUM- BER	YEAR	TOTAL	WATER- SOLUBLE	(1) A.O.A.C. BATH; PERIODIC AGITATION*	BY ELECTRIC OVEN; CONSTANT AGITATION 1 HOUR AT VARIANT TEMPERATURES					CITRATE-INSOLUBLE					VARIANCE FROM (1) BY 1-HOUR CONSTANT AGITATION AT VARIANT TEMPERATURES				
							30°C.	40°C.	50°C.	60°C.	65°C.	30°C.	40°C.	50°C.	60°C.	65°C.	30°C.	40°C.	50°C.	60°C.	65°C.
Superphosphate	—	A-1	1931	20.44	17.17	0.03	0.75	0.41	0.14	0.04	0.008	+0.72	+0.38	+0.11	+0.01	-0.022					
Ammoniated superphosphate	2.48	A-10	1931	20.63	12.61	1.97	3.10	2.45	2.15	1.95	1.72	+1.13	+0.48	+0.18	-0.02	-0.25					
Ammoniated superphosphate	7.35	A-9	1931	18.79	1.53	6.35	8.20	6.95	6.65	6.35	6.15	+1.85	+0.60	+0.30	0.00	-0.20					
Ammoniated superphosphate	8.66	A-13	1931	19.25	0.20	6.93	9.55	8.20	7.75	7.35	6.85	+2.62	+1.77	+0.87	+0.42	-0.08					
Dicalcium phosphate†	—	2	1936	51.75	0.15	2.60	18.25	12.55	6.60	4.65	1.98	+15.65	+9.95	+4.00	+2.05	-0.62					
Calcined phosphate	—	10	1936	36.88	0.05	3.65	6.90	5.15	4.30	3.70	3.48	+3.25	+1.50	+0.65	+0.05	-0.17					
Calcium metaphosphate	—	13	1936	61.88	5.65	0.08	4.88	0.93	0.11	0.08	0.06	+4.80	+0.85	+0.03	0.00	-0.02					
Fused phosphate	—	14	1936	26.79	0.11	3.75	8.75	6.60	4.45	3.45	2.95	+5.00	+2.85	+0.70	-0.30	-0.80					
Mixed fertilizer (6-9-4)	4.00	6	1935	9.88	0.67	0.79	1.15	1.10	1.00	0.85	0.75	+0.36	+0.31	+0.21	+0.06	-0.04					
Number of plus values												9	9	9	5						
Mean of plus values												3.93	2.08	0.78	0.52						
Number of minus values															4						
Mean of minus values															0.08	0.24					

\* At 5-minute intervals.

† This product was not a true CaHPO<sub>4</sub>.

TABLE 3.—*Effect of duration of 65°C. citrate digestion with continuous agitation in the determination of citrate-insoluble  $P_2O_5$  (per cent)*

A.O.A.C. REFERENCE SAMPLE				P <sub>2</sub> O <sub>5</sub> VALUES											
TYPE	NH <sub>3</sub> CONTENT	NUMBER	YEAR	TOTAL	WATER SOLUBLE	CITRATE-INSOLUBLE					VARIANCE BY CONSTANT AGITATION				
						A.O.A.C. BATH; PERIODIC AGITATION*	BY ELECTRIC OVEN; CONSTANT AGITATION								
							15 MIN.	30 MIN.	45 MIN.	60 MIN.	15 MIN.	30 MIN.	45 MIN.	60 MIN.	
Superphosphate	—	A-1	1931	20.44	17.17	0.03	0.18	0.04	0.015	0.008	+0.12	+0.01	-0.015	-0.022	
Superphosphate	—	A-2	1931	20.13	12.28	0.02	0.05	0.03	0.01	0.016	+0.03	+0.01	-0.01	-0.004	
Ammoniated superphosphate	2.48	A-10	1931	20.63	12.16	1.97	2.23	1.83	1.75	1.72	+0.26	-0.14	-0.22	-0.25	
Ammoniated superphosphate	7.35	A-9	1931	18.79	1.53	6.35	6.88	6.65	6.20	6.15	+0.53	+0.30	-0.15	-0.20	
Ammoniated superphosphate	8.66	A-13	1931	19.25	0.20	6.93	8.05	7.25	6.98	6.85	+1.12	+0.32	+0.05	-0.08	
Ammoniated superphosphate	5.65	3	1938	41.38	2.38	2.40	2.50	2.44	2.50	2.40	+0.10	+0.04	+0.10	0.00	
Dicalcium phosphate†	—	2	1936	51.75	0.15	2.60	11.38	5.68	3.82	1.98	+8.78	+3.08	+1.22	-0.62	
Calcined phosphate	—	10	1936	36.88	0.05	3.56	4.38	3.78	3.70	3.43	+0.73	+0.13	+0.05	-0.17	
Calcined phosphate (reverted)	—	11	1936	36.63	0.03	19.70	22.40	20.90	19.88	19.63	+2.70	+1.20	+0.18	-0.07	
Imported basic slag	—	12	1936	17.13	0.03	3.01	3.58	3.03	2.85	2.80	+0.57	+0.02	-0.16	-0.21	
Fluorspar basic slag	—	B-11	1931	10.75	0.14	9.25	9.45	9.13	9.25	9.25	+0.20	-0.12	0.00	0.00	
Calcium metaphosphate	—	13	1936	61.88	5.65	0.08	0.53	0.08	0.07	0.06	+0.45	0.00	-0.01	-0.02	
Fused phosphate	—	14	1936	26.79	0.11	3.75	6.07	4.10	3.20	2.95	+2.32	+0.35	-0.55	-0.80	
Phosphate rock (B.S. 120)	—	—	—	35.31	—	32.19	32.69	32.13	32.00	31.88	+0.50	-0.06	-0.19	-0.31	
Mixed fertilizer (5-10-5)	5.00	2	1938	15.88	8.35	4.88	5.00	4.88	4.80	4.73	+0.12	0.00	-0.08	-0.15	
Number of plus values											15	10	5		
Mean of plus values											1.23	0.55	0.32	15	
Number of minus values												5	10	15	
Mean of minus values												0.06	0.12	0.19	

\* At 5-minute intervals.

† This product was not a true CaHPO<sub>4</sub>.

TABLE 4.—Comparison of 30- and 60-minute digestions with continuous agitation in determination of citrate-insoluble  $P_2O_5$  (per cent)

A.O.A.C. REFERENCE SAMPLE				P <sub>2</sub> O <sub>5</sub> VALUES						
TYPE	NH <sub>4</sub> CONTENT	NUMBER	YEAR	TOTAL	WATER-SOLUBLE	CITRATE-INSOLUBLE				
						A.O.A.C. BATH; PERIODIC AGITATION <sup>a</sup>	BY ELECTRIC OVEN; CONSTANT AGITATION		VARIANCE BY CONSTANT AGITATION	
							30 MIN.	60 MIN.		30 MIN.
Superphosphate	—	A-1	1931	20.44	17.17	0.03	0.04	0.008	+0.01	-0.022
Superphosphate	—	A-2	1931	20.13	12.28	0.02	0.03	0.016	+0.01	-0.004
Superphosphate	—	A-4	1931	18.75	6.88	0.06	0.12	0.03	+0.06	-0.03
Triple superphosphate	—	3	1935	47.50	36.81	2.99	2.98	2.95	-0.01	-0.04
Ammoniated superphosphate	2.48	A-10	1931	20.63	12.61	1.97	1.03	1.72	-0.14	-0.25
Ammoniated superphosphate	4.36	A-15	1931	19.37	8.75	2.75	2.90	2.60	+0.15	-0.15
Ammoniated superphosphate	4.71	A-14	1931	19.28	9.25	2.88	3.10	2.80	+0.22	-0.08
Ammoniated superphosphate	5.36	A-11	1931	20.00	9.63	2.45	2.25	2.25	+0.25	-0.20
Ammoniated superphosphate	7.35	A-9	1931	18.79	1.53	6.35	6.65	6.15	+0.30	-0.20
Ammoniated superphosphate	8.66	A-13	1931	19.25	0.20	6.93	7.25	6.85	+0.32	-0.08
Ammoniated superphosphate	5.65	3	1938	41.38	2.38	2.40	2.44	2.40	+0.04	0.00
Ammoniated superphosphate	5.65	4	1938	41.19	3.59	4.58	4.23	4.42	+0.35	-0.16
Limited superphosphate	—	A-5	1931	17.12	0.55	4.18	4.20	3.68	+0.02	-0.50
Monocalcium phosphate	—	1	1936	56.32	54.25	0.00	0.00	0.00	0.00	0.00
Dicalcium phosphate	—	2	1936	51.75	0.15	2.60	5.68	1.98	+3.08	-0.62
Calcined phosphate	—	4	1936	37.06	0.04	7.54	8.08	4.98	+0.54	-2.56
Calcined phosphate	—	10	1936	36.88	0.05	3.65	3.78	3.48	+0.13	-0.17
Calcined phosphate (reverted)	—	11	1936	36.63	0.03	19.70	20.90	19.63	+1.20	-0.07
Imported basic slag	—	12	1936	17.13	0.03	3.01	3.03	2.80	+0.02	-0.21
Fluorspar basic slag	—	B-11	1931	10.75	0.14	9.25	8.50	9.25	-0.75	0.00
Calcium metaphosphate	—	13	1936	61.88	5.65	0.08	0.08	0.06	0.00	-0.02
Fused phosphate	—	14	1936	26.79	0.11	3.75	4.10	2.95	+0.35	-0.80
Bone ash	—	B-12	1931	40.75	0.42	35.13	35.25	35.00	+0.12	-0.12
Phosphate rock (B.S. 120)	—	—	—	35.31	—	32.19	32.13	31.88	-0.06	-0.31
Mixed fertilizer (6-8-4)	4.00	5	1935	21.96	4.35	13.55	13.55	13.28	+0.20	-0.07
Mixed fertilizer (6-9-4)	4.00	6	1935	9.88	0.67	0.79	0.88	0.75	+0.09	-0.04
Mixed fertilizer (3-10-5)	5.00	4	1935	14.88	3.55	4.68	4.85	4.62	+0.17	-0.08
Mixed fertilizer (5-10-5)	5.00	2	1938	15.88	0.35	4.88	4.88	4.73	0.00	-0.15
Mixed fertilizer (6-12-6)	6.00	1	1938	18.07	2.68	3.45	3.83	3.40	+0.38	-0.05
Number of plus values									21	29
Mean of plus values									8	8
Number of minus values									0.26	0.25
Mean of minus values										

<sup>a</sup> At 5-minute intervals.<sup>b</sup> This product was not a true CaHPO<sub>4</sub>.

The 40, 50, 60, and 65°C. temperatures refer to those of the digestates, rather than to the atmosphere of the chamber. Introduced into the chamber and then not agitated, digestates attain the temperature of the chamber atmosphere, and that temperature persists so long as the digestate is quiescent. Upon agitation, the digestate shows a rise in temperature and the atmosphere of the chamber shows a decrease. This was observed by window-reading of the Anschütz thermometers, the bulbs of which were submerged when the flasks were at rest in upright position. When the Anschütz thermometers registered digestate temperatures of 40, 50, 60, and 65°C. for separate digestions, the corresponding simultaneous readings of chamber atmosphere were 39½, 45, 58, and 63°C. During the digestions with agitation at 65°C., the chamber atmosphere registered 63°C., and that temperature was maintained one week by an electric thermoregulator. This relationship between the temperatures of digestate and chamber atmosphere is reached quickly and continues regardless of laboratory temperature and convective currents. In contrast, when the citrate is brought to the prescribed 65°C. in a water bath and the washed analytical residue then is introduced, the solution does not again come to 65°C. during an hour's digestion.

#### VARIATION IN DIGESTION PERIOD WITH CONSTANT AGITATION

One objective was to determine whether continuous agitation in the heated chamber would admit of briefing of digestion at 65°C. The samples of Tables 1 and 2 and several additional materials were used in the comparisons of Table 3. These comparisons show that, in general, increase of time induced lower C.I. values. All of the C.I. values by continuous agitation for the minimal 15-minute period were higher than those obtained by the official procedure. For some products, the acidic materials and calcium metaphosphate in particular, the 30-minute agitation was as effective as the 60-minute requirement of the official digestion. Again, with lone exception of the recalcitrant anhydrous dicalcium phosphate, the continuous agitation for 45 minutes was as effective as the 1-hour digestion with periodic agitation. Without exception, every 60-minute digestion with continuous agitation gave a C.I. value either lower than or identical with the one obtained by the official procedure.

In the digestions of the 29 variant phosphatic materials (Table 4), a variance of 0.15 per cent or less from the official method values was registered by the 30-minute digestions in 16 instances, whereas eleven of the digestions gave plus variances beyond 0.2 per cent. Although the continuous agitation during 30 minutes of digestion was as effective as an additional period of 30 minutes with periodic agitation for most of the commercial fertilizers, again the exceptions were such as to preclude adoption of the 30 minute period. Once more, every citrate-insoluble value obtained



by 60 minutes of agitated digestion was less than the corresponding value obtained by the official procedure.

### SUMMARY

It was demonstrated that a variance in spot temperatures occurs in the water of a citrate-digestion bath and in the lagging temperatures of the contained digestates. Parallel digestions were conducted in an electrically-heated water bath and in an electrically-heated chamber, well insulated; and provided with a device for constant agitation of digestates. The chamber afforded greater uniformity of temperature, and induced greater dissolvent action and better reproducibility of citrate-insoluble values than did the official technic for the same digestion period.

The dissolvent action of the citrate increased progressively during the 1-hour digestions at temperatures of 30, 40, 50, 60, and 65°C. Chamber digestions with constant agitation for periods of 15 minutes and 30 minutes proved ample in some instances and inadequate in others. With one exception, a 45-minute digestion was ample when the digestates were agitated continuously. The citrate-insolubility values obtained by constant agitation were invariably lower than those obtained by the official procedure.

The proposed home-made digestion chamber meets the official requirements. It assures uniformity in spot temperature, eliminates variations in manual agitation, and conserves the time and attention of the analyst. It improves reproducibility of analytical results, is clean and inexpensive to operate, and is ever-ready.

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### MODIFICATIONS OF A.O.A.C. CHICK METHOD OF VITAMIN D ASSAY\*

By ROBERT JOHN EVANS and J. L. ST. JOHN (Division of Chemistry,  
Washington Agricultural Experiment Station, Pullman, Wash.)

The A.O.A.C. chick method for estimating vitamin D<sup>1</sup> is time consuming and laborious. Any modification which would decrease the time and labor required without also decreasing the accuracy of the method would be of great value.

The assay period can not be shortened to less than the 21 days specified without decreasing the accuracy of the method.<sup>2</sup> The cleaning of the bones is time consuming. Johnson<sup>3</sup> suggested that the removal of the epiphyseal cartilages from the tibiae of the chick would facilitate rapid and complete removal of adhering tissue from the bone. Fritz and Halloran<sup>4</sup> presented data substantiating the work of Johnson,<sup>3</sup> but they suggested that boiling the legs for four minutes in hot water would be as effective in speeding cleaning of the bones. St. John, Kempf, and Bond,<sup>5</sup> advocated the separation of the shaft from the cartilage and suggested that cartilage ash might be a better criterion of the effectiveness of vitamin D supplements than the ash of the entire tibia. Baird and MacMillan<sup>6</sup> advocated the use of toes rather than tibiae for ashing to save time and chicks.

It appeared to the authors that further work was desirable to determine the relative value of ashing different portions of the tibiae and the toes for the A.O.A.C. chick vitamin D assay method.<sup>1</sup> This paper presents such a study.

### EXPERIMENTAL

During a study to determine the value of fish oils fortified with syn-

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<sup>1</sup> *Methods of Analysis, A.O.A.C.*, 1940, 371.

<sup>2</sup> DeWitt, J. B., C. D. Tolle, H. W. Loy, Jr., and L. F. Knudsen, *This Journal*, **25**, 213 (1942).

<sup>3</sup> *Poultry Sci.*, **21**, 329-332 (1942).

<sup>4</sup> *Ibid.*, **22**, 314-322 (1943).

<sup>5</sup> *Ibid.*, **12**, 84-86 (1933).

<sup>6</sup> *This Journal*, **25**, 518-524 (1942).

thetic vitamin D, a reference cod liver oil containing 115 A.O.A.C. chick units of vitamin D per gram was fed to duplicate groups of 15 chicks per group at levels of 0, 5, 10, and 15 units per 100 grams of diet. Five fish oils were also fed to other groups at approximate levels of 5, 10, and 15 units per 100 grams of diet. Day-old White Leghorn cockerels were used and were sacrificed after three weeks on the experimental diets. The left tibia was utilized in determining the value of fortification with synthetic D. In addition the middle toes of the right and left feet were cut off as described by Baird and MacMillan.<sup>6</sup> The right, as well as the left, tibia was taken as described in the official A.O.A.C. method. The right tibia was divided into three parts: the shaft, the proximal cartilage, and the distal cartilage. These and the middle toe of the left foot were extracted with alcohol and ether as recommended in the official method. The middle toe of the right foot was not extracted. These were used for the comparisons presented in the present paper. The results of the synthetic D fortifications study are presented elsewhere by Evans, Rhian, Carver, Hamm, and Harrison.<sup>7</sup>

The bones were ashed by groups for one hour at 850° C.

## RESULTS

Percentages of ash in the toes and tibiae of 3-weeks-old chicks receiving 0, 5, 10, and 15 A.O.A.C. chick units of vitamin D per 100 grams of diet are presented in Table 1. The values for the right tibia were obtained by

TABLE 1.—*Per cent ash in tibiae and toes of chicks receiving graded levels of supplementary vitamin D in diet*

GROUP NO.	LEVEL VIT. D	LEFT TIBIA	RIGHT TIBIA	SHAFT RIGHT TIBIA	PROXIMAL CARTILAGE	DISTAL CARTILAGE	LEFT TOE	UNEXTRACTED RIGHT TOE
1	0	34.0	33.8	41.3	5.0	12.1	11.5	11.7
23		33.6	33.0	39.3	4.8	12.3	11.0	11.1
Av.		33.8	33.4	40.3	4.9	12.2	11.3	11.4
2	5	36.5	36.2	43.8	4.8	13.9	12.9	12.6
14		35.1	34.5	41.0	4.8	12.9	12.3	12.5
Av.		35.8	35.4	42.4	4.8	13.4	12.6	12.6
6	10	39.2	39.3	47.0	5.6	15.7	13.9	13.9
18		38.7	38.0	45.7	4.9	14.7	12.9	12.8
Av.		39.0	38.6	46.4	5.2	15.2	13.4	13.4
10	15	40.6	40.5	47.1	4.6	16.1	14.6	14.4
22		42.9	42.7	48.8	4.4	17.5	15.0	14.4
Av.		41.8	41.6	48.0	4.5	16.8	14.8	14.4

<sup>7</sup> *Poultry Sci.*, 23, 91 (1944).

adding the weight of ash from the shaft, proximal cartilage, and distal cartilage, and dividing the sum by the weight of the whole tibia. Differences between the right and left tibia are probably due to experimental error. The shaft contained the highest percent of ash at a given level of vitamin D. The proximal cartilage contained the least ash. The vitamin D content of the diet appeared to have no influence on the ash of the proximal cartilage at this age, although an effect was found at eight weeks of age by St. John, Kempf, and Bond (1933).<sup>5</sup> The toes and distal cartilage

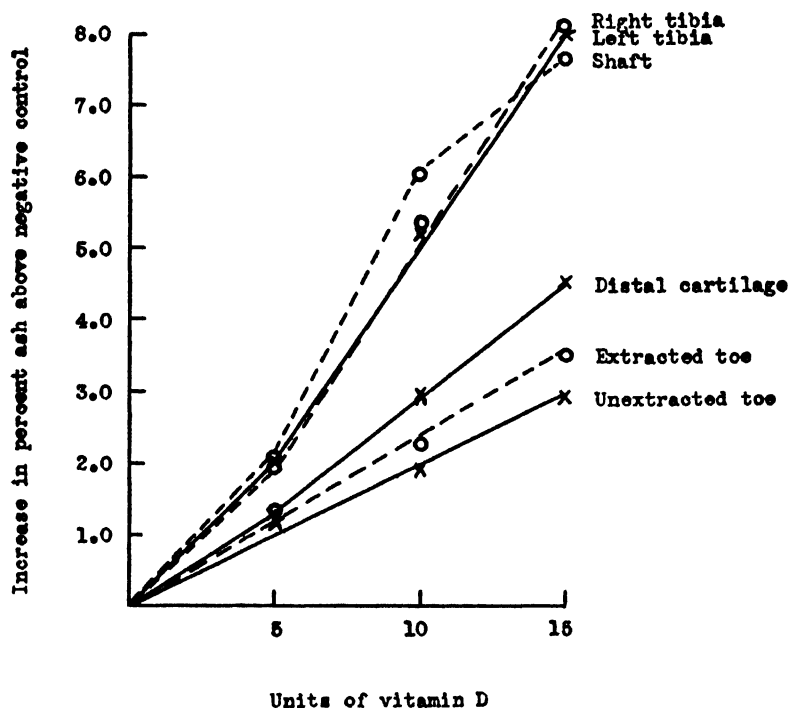


FIG. 1.—INCREASE IN PERCENT ASH ABOVE NEGATIVE CONTROL OF BONES AND TOES OF CHICKS RECEIVING ADDED LEVELS OF VITAMIN D IN THE DIET.

contained much the same percentages of ash as shown in Table 1. Figure 1 gives the increase in bone ash above the negative control caused by the addition of vitamin D to the diet.

To determine the sensitivity of the different methods the increase in bone ash above the negative control was divided by the bone ash of the negative control. The resulting values, expressed as percent increase, are plotted against units of added vitamin D in Figure 2. The distal cartilage was the most sensitive since the increase per unit of added vitamin D was greatest. The shaft was the least sensitive, except the proximal cartilage, which gave no indication of the vitamin D content. The values for the

shaft were irregular, and they are not included in this figure. Both the unextracted right toe and the extracted left toe were slightly more sensitive than the tibia.

All the curves in Figures 1 and 2 have been drawn through the zero point. If the curves for the right and left tibia and for the distal cartilage

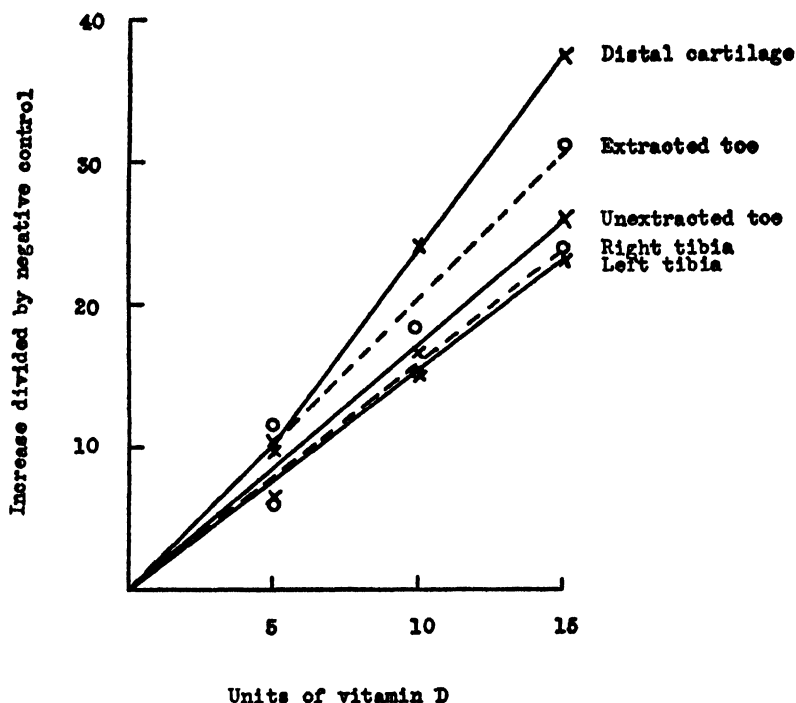


FIG. 2.—RELATIVE SENSITIVITY OF METHODS OF VITAMIN D ASSAY USING PER CENT ASH IN TOES AND TIBIAE. SENSITIVITY WAS DETERMINED BY DIVIDING THE INCREASE IN PER CENT BONE ASH ABOVE THE NEGATIVE CONTROL BY THE PER CENT BONE ASH OF THE NEGATIVE CONTROL AND WAS EXPRESSED AS A PERCENTAGE.

are extrapolated from the three experimental points they tend to converge on the abscissa at a value of about 3 rather than zero in Figure 1.

An indication of the relative accuracy or experimental error of the methods can be obtained by the difference between duplicate groups. Dividing the differences by the total ash gave values which varied little for the methods used, indicating small difference in experimental accuracy.

The value of any procedure is dependent on the results obtained. Table 2 presents the unitage of vitamin D in each of five samples of fish oil calculated from the bone ash determinations on the toes, the tibia, and each of the tibia fractions. In calculating the average values for the sockeye salmon oil, the values obtained at the 5 unit level were discarded, since all were low compared to the other levels.

TABLE 2.—*Vitamin D content of fish oil as determined by use of per cent ash in toes and different fractions of tibiae. (A.O.A.C. chick units vitamin D/gram oil)*

GROUP NO.	KIND OF OIL	APPROX. LEVEL OIL	LEFT TIBIA (OFFICIAL)	RIGHT TIBIA	SHAFT RIGHT TIBIA	DISTAL	LEFT TOE	RIGHT TOE UNEXT.
3	Herring	5	200	180	125	220	160	240
4		10	141	165	140	188	155	190
5		15	237	257	235	253	260	240
Av.			193	201	167	220	192	223
7	Pilchard	5	156	212	312	352	232	244
8		10	158	180	160	176	168	168
9		15	165	171	163	141	147	147
Av.			160	188	212	223	182	186
11	Dogfish liver	5	248	248	167	230	225	153
12		10	180	173	144	180	214	198
13		15	180	189	176	186	188	213
Av.			203	203	162	199	209	188
15	Pink salmon	5	163	155	130	128	200	170
16		10	193	178	161	173	188	201
17		15	160	154	146	148	151	163
Av.			172	162	146	150	180	178
19	Sockeye salmon	5	(95)	(100)	(73)	(108)	(70)	(73)
20		10	159	161	140	144	103	179
21		15	168	157	149	148	136	136
Av.*			163	159	145	146	120	158

\* The results obtained at the 5 unit level were not used in calculating the average.

The average values for vitamin D as assayed by the different methods, the differences from the left tibia, and the per cent difference are presented in Table 3. The poorest agreements were obtained by using the shaft and the distal cartilage. The average per cent difference is very low with the extracted left toes, but since the value for the sockeye salmon was low, the standard deviation was 15. Both the average difference and the standard deviation were slightly higher for the unextracted right toe than for the right tibia, which can be considered as a duplicate of the left tibia.

#### DISCUSSION

The use of the toes of chicks rather than the tibia, as advocated by Baird and MacMillan,<sup>6</sup> is an important step in the simplification of the chick assay method for vitamin D. They used the extracted toes but suggested that use of the unextracted toes might be satisfactory. Results presented in this paper indicate that the ash content of either the extracted or unextracted toes gives a satisfactory assay of the vitamin D

content of a fish oil. The use of toes greatly decreases the time and labor required in the A.O.A.C. assay procedure. Since unextracted toes can be used and results obtained similar to those obtained with extracted toes, extraction of the left tibia before ashing in the official method may not be necessary. This has been shown to be the case by Fritz and Halloran.<sup>4</sup>

TABLE 3.—*Comparison of vitamin D potencies obtained by ashing different bones in the assay procedure for fish oils*

BONE USED	HERRING	PILCHARD	DOGFISH	PINK SALMON	SOCKEYE SALMON	AV. $\pm$ DIFF.	ALGEBRAIC AVERAGE	STANDARD DEVIATION
Left tibia (official)	193	160	203	172	163			
Right tibia	201	188	203	162	159			
Difference	8	28	0	-10	-4			
Per cent diff.	4	18	0	-6	-2	6	3	9
Shaft	167	212	162	146	145			
Difference	-26	52	-41	-26	-18			
Per cent diff.	-13	33	-20	-17	-11	18.8	-6	22
Distal cartilage	220	223	199	150	146			
Difference	27	63	-4	-22	-17			
Per cent diff.	14	39	-2	-13	-10	15.6	6	21
Left toe (extracted)	192	182	209	180	120			
Difference	-1	22	6	8	-43			
Per cent diff.	-1	14	3	5	-25	9.6	-1	15
Right toe (unextracted)	223	116	188	178	158			
Difference	30	26	-15	6	-5			
Per cent diff.	16	16	-7	3	-3	9.0	5	11

The data presented in this paper, while supporting the suggestion of Johnson<sup>3</sup> and of Fritz and Halloran<sup>4</sup> that the shaft can replace the tibia in the A.O.A.C. ashing procedure, do not show that results obtained with the shaft will be as satisfactory as those obtained with the whole tibia. In the present study this was probably caused by difficulty in separating the shaft from the cartilage.

#### SUMMARY

The middle toe of the chick, extracted with alcohol and ether, can be satisfactorily used to replace the tibia for ashing in the A.O.A.C. chick vitamin D assay method, corroborating the work of Baird and MacMillan.<sup>6</sup> The unextracted toe can be used equally well. The toes are slightly more sensitive than the tibiae, and the procedure is also much more rapid and makes unnecessary the sacrifice of the chicks at the conclusion of the assay.

The per cent ash in the distal cartilage gave the most sensitive response to added levels of vitamin D, while the percent ash in the shaft gave the least, supporting the results of St. John, Kempf, and Bond.<sup>5</sup> At three weeks of age the proximal cartilage showed no increase in percent ash resulting from increased vitamin D in the diet. Values obtained for the vitamin D content of fish oils by the use of per cent ash in the shaft, distal cartilage, extracted toe, or unextracted toe compare favorably with values obtained by using the per cent ash in the tibia.

### USE OF TURKEYS FOR ASSAY OF VITAMIN D\*

By G. B. WILLGEROTH, J. L. HALPIN, H. R. HALLORAN; and J. C. FRITZ  
(The Borden Company, Nutritional Research Laboratory,  
Elgin, Ill.)

Collaborative studies with the chick assay for vitamin D indicate serious need for improved accuracy. Summarizing such studies, Baird and Barthen (1) conclude in part, "An experimental animal that will react more uniformly is needed, and this is thought possible to achieve only by developing a strain of chicks with a high vitamin D requirement which will respond proportionately to graded doses of vitamin D." Similar conclusions are noted in the more recent work of Motzok, Hill, Branion, and Slinger (2), who recommend the use of hybrid chicks.

Data accumulated by Scott, Hughes, and Loy (3), Baird and Greene (4), Jukes and Sanford (5), Hammond (6), and Carver and Rhian (7) indicate that turkey poults possess many of the desired characteristics. Jukes and Sanford (5) suggested the turkey as a sensitive test animal for the study of vitamin D, but they questioned whether a "turkey unit" was an accurate measure of the vitamin D activity for chickens.

Test work was planned at this laboratory to determine the requirement of turkey poults for vitamin D and the range in bone ash under assay conditions. Furthermore, the study was designed to compare the effectiveness of different compounds having vitamin D activity, and to compare the results of assays conducted with chicks and with poults.

### MATERIALS AND METHODS

Bronze turkey poults hatched from the laboratory flock were used in this study. The chicks used were Single Comb White Leghorns, also hatched from the laboratory flock. The number of poults used per group varied from 5 to 12. The chick work represented groups of 20 birds each.

Both chicks and poults were kept under usual conditions in screen-floored, chick-starting batteries. Table 1 shows the composition of the

\* A preliminary report was presented before the American Chemical Society, Division of Agricultural and Food Chemistry, Pittsburgh, Penn., September 6-10, 1943.



TABLE 1.—*Composition of diets*

INGREDIENTS	POULTS per cent	CHICKS per cent
Ground yellow corn	48	77
Acid-washed casein	15	12
Wheat bran	10	5
Wheat standard middlings	10	—
Dried skim milk	5	—
Dehydrated alfalfa	5	—
Non-irradiated yeast	2	2
Tricalcium phosphate	2	2
Meat and bone scrap	1	1
Oyster shell flour	1	—
Iodized salt with 5% $MnSO_4$	1	1
	100	100
Protein	24.73	19.02
Calcium	1.66	0.93
Phosphorus	1.06	0.87

diets used. In all cases the birds received the designated diet and fresh tap water *ad libitum*. Except as noted, both chicks and poults were handled in accordance with the A.O.A.C. vitamin D method (8).

#### EXPERIMENTAL

Most of the data in the literature indicate that turkey poults require about 70 or 80 A.O.A.C. units of vitamin D per 100 grams of feed for optimum calcification (4, 6, 7). Jukes and Sanford (5), however, found a requirement approximately two and one-half times as high. The reason for the unusually high requirements of their poults has not been satisfactorily explained.

Groups of twelve poults each were fed graded levels of vitamin D for eight weeks in an effort to determine the requirements under conditions in this laboratory. The data are summarized in Table 2. In this test approximately 70 units per 100 grams of feed gave maximum calcification.

TABLE 2.—*Vitamin D requirements*

A.O.A.C. UNITS VITAMIN D/100 GRAMS	AVERAGE WT. OF POULTS AT 8 WEEKS grams	BONE ASH AT 8 WEEKS per cent
25	Terminated at 3 weeks—severe rickets	
40	692	41.40
55	936	47.01
70	944	51.62
100	961	49.01

Table 3 shows the response of comparable groups of poults to graded levels of U.S.P. reference cod liver oil and to different compounds having

vitamin D activity. Chick assays were available to provide data on the activated 7-dehydrocholesterol which was dissolved in vegetable oil. The vitamin D<sub>2</sub> was a vegetable oil solution of activated ergosterol which had been carefully assayed by repeated rat tests (9). The dihydrotachysterol was a sesame oil solution obtained from a reliable company. McClesney (10) found 1 mg. of dihydrotachysterol to have an anti-rachitic potency equivalent to 80 U.S.P. units, or to 360 A.O.A.C. units of vitamin D. For the purposes of this test a slightly higher potency was assumed.

This test was of three weeks' duration, and individual weights and bone ash data were obtained. The standard deviations of the data are shown

TABLE 3.—*Response of turkey poult to Vitamin D*

SUPPLEMENT	ESTIMATED POTENCY		SUPPLEMENT FED/100 GRAMS FEED	WT. AT 3 WEEKS		BONE ASH	
	PER GRAM SUPPLE- MENT	PER 100 GRAMS FEED		Av.	S.D.	Av.	S.D.
			mg.	grams		per cent	
None	0	0	0	152	8	26.77	1.24
U.S.P. Ref. C.L.O. No. 2	115	15	130.4	185	23	28.51	0.17
U.S.P. Ref. C.L.O. No. 2	115	30	260.9	250	20	34.79	3.83
U.S.P. Ref. C.L.O. No. 2	115	45	391.3	219	14	36.34	2.61
U.S.P. Ref. C.L.O. No. 2	115	60	521.7	215	54	41.85	1.52
U.S.P. Ref. C.L.O. No. 2	115	75	652.2	211	27	46.09	0.99
D <sub>2</sub> in oil	6,425	45	7.004	182	56	36.66	1.60
D <sub>2</sub> in oil	400,000*	75*	0.1875	120	13	25.51	0.89
D <sub>2</sub> in oil	400,000*	750*	1.875	132	23	27.68	2.17
Dihydrotachysterol	450,000	45	0.1000	238	40	47.62	3.21

\* U.S.P. units. Other estimated potencies are in terms of A.O.A.C. units.

in Table 3 to provide a measure of the variability within groups. The standard deviations from the mean per cent bone ash for two groups of chicks were 3.16 and 2.50, respectively. It is, therefore, obvious that the variation between individual poult within a group is no greater than between individual chicks within a group.

The activity of vitamin D<sub>2</sub> was essentially the same as for chicks. The activity of vitamin D<sub>2</sub> for poult was calculated to be only 1.3 per cent of that for rats. This, too, is within the range usually found in chick tests with vitamin D<sub>2</sub>. The dihydrotachysterol showed an activity much greater than was anticipated. Apparently poult utilize this sterol more efficiently than do either chicks or rats. It is impossible from these data to assign exact figures for the activity of dihydrotachysterol.

The percentage of bone ash as influenced by the vitamin D intake varies over about twice the range commonly observed with chicks. The data show that an intake of 45 A.O.A.C. units of vitamin D per 100 grams of

feed produces a bone ash level which falls on the steep part of the response curve, and that this should be a satisfactory assay level for poults. In routine chick assays at this laboratory, a level of 15 A.O.A.C. units of vitamin D per 100 grams of feed is ordinarily used.

A comparison of simultaneous assays with chicks and poults is summarized in Table 4. Unfortunately, the agreement between duplicate pens

TABLE 4.—*Comparison of assays with chicks and poults*

SUPPLEMENT DESCRIPTION	ESTIMATED POTENCY/ GRAM	CHICKS			POULTS		
		SUPPLE- MENT/100 GRAMS FEED	AVERAGE BONE ASH	UNITS/ GRAM	SUPPLE- MENT/100 GRAMS FEED	AVERAGE BONE ASH	UNITS/ GRAM
Negative Control	—	mg. —	per cent 32.09	—	mg. —	per cent 23.97	—
U.S.P. Ref. C.L.O. No. 2	115	43.5	35.07	115	260.9	35.38	115
U.S.P. Ref. C.L.O. No. 2	115	87.0	36.78	115	391.3	37.93	115
U.S.P. Ref. C.L.O. No. 2	115	87.0	37.84	115	521.7	44.80	115
U.S.P. Ref. C.L.O. No. 2	115	130.4	39.40	115			
U.S.P. Ref. C.L.O. No. 2	115	173.9	40.38	115			
A and D Feeding Oil	400	37.5	39.37	400	112.5	46.13	540
A and D Feeding Oil	400	37.5	40.28	530			
Yellow Fin Tuna	16,000	0.9375	36.79	9,500	2.8125	37.24	13,700
Liver Oil	16,000	0.9375	37.48	11,000			
Bonito-Skipjack	2,000	7.5	40.31	2,667	22.5	40.08	2,310
Liver Oil	2,000	7.5	39.80	2,200			
D-activated Animal Sterol	200,000	0.0750	40.12	257,000	0.2250	43.50	260,000
Fish Liver	85	176.4	40.92	>115	529.4	47.60	>115

of chicks was not as good as might be desired. It does, however, indicate the variations which may be expected in the use of this method. It will be noted that the turkey data on the A and D feeding oil agree closely with the higher results obtained in the chick assays. In the case of the yellow fin tuna liver oil, slightly higher results were obtained with the poults, but all results were below the estimated potency. The lower chick results on the bonito-skipjack liver oil sample agree within reasonable limits with the turkey data. Practically identical results were obtained on the D-activated animal sterol. The potency of a sample of fish liver was underestimated, and results above the range of the reference curve were obtained with both species.

Figure 1 shows a comparison of the two response curves obtained in this assay.

It should be noted that during these studies severe rickets developed in poults fed a diet lacking in vitamin D but otherwise believed to be ade

quate. The levels and ratio of calcium and phosphorus were considered satisfactory for growth and calcification. Such results are not always obtained in chick tests when the mineral balance is adjusted for optimum calcification (11, 12, 13). A group of chicks was fed the turkey basal diet

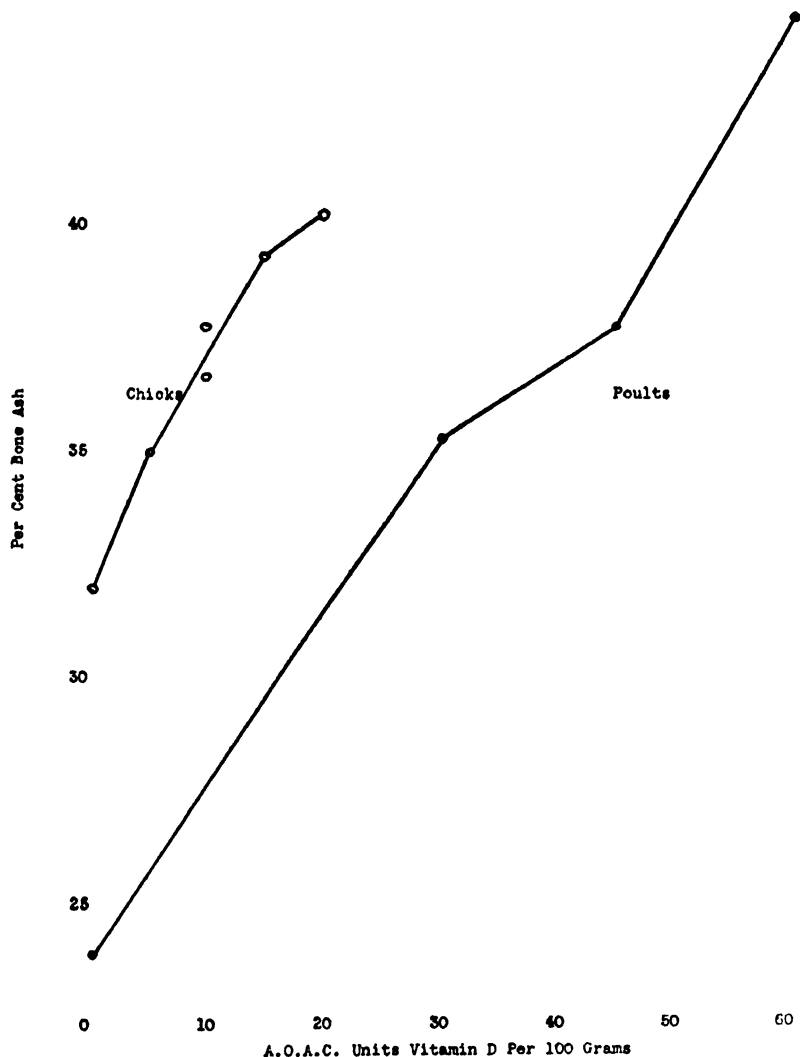


FIG. 1.—COMPARISON OF RESPONSE CURVES.

without vitamin D. At the end of three weeks these chicks were found to contain 36.58 per cent of ash in the dry, fat-free tibiae, while the poults on the same program had 23.97 per cent of ash in the dry, fat-free tibiae. This indicates that the poult would be a much more satisfactory test

animal for the assay of low potency samples containing minerals. In such cases, the poults would offer a reliable measure of the amount of vitamin D, whereas the chicks would measure vitamin D less accurately because their calcification would be influenced by the mineral content of the sample.

#### DISCUSSION

The higher requirements, the greater range in bone ash due to varying vitamin D intake, and the lesser influence of factors other than vitamin D should make the poult more satisfactory than the chick for the assay of vitamin D.

Simultaneous assays with these two species usually indicate similar potencies for the material tested. Some few samples show a greater activity when tested with poults. Since similar activity of vitamin D<sub>2</sub> and vitamin D<sub>3</sub> is observed, it seems probable that any differences in results must be due to other sterols. We have seen that dihydrotachysterol is more effective on poults, and it is quite possible that there may be other compounds which do not have identical anti-rachitic activity for the two species. It is believed, however, that for most samples an assay with either species affords a reliable indication of its anti-rachitic potency for the other species.

Growth of the poults during the three-week test did not parallel the bone ash values, and the weight of the birds could not be used as a criterion of vitamin D intake. Other tests indicate that there may be a correlation between body weights and vitamin D intake when the poults are carried beyond three weeks of age. Individual variation within groups was about the same as within groups of chicks. This applied to both weight and bone ash data. In the case of the bone ash data this variation is believed to be less serious in the poults because of the wider range due to varying vitamin D intake.

Disadvantages of the use of poults for vitamin D assay would consist chiefly in the lack of a year-around supply, and the higher cost as compared with chicks. It is probable that the first disadvantage could be at least partially overcome by selection of the breeding stock if there was any incentive for such selection. Differences in cost might be eliminated if equal accuracy could be obtained with a smaller number of poults. In view of the data presented, it is believed that the advantages in the use of poults outweigh the disadvantages, and that this species is deserving of consideration for the vitamin D assay of poultry feed supplements.

#### SUMMARY

Turkey poults require at least 70 A.O.A.C. units of vitamin D per 100 grams of feed for optimum calcification. The bone ash range with varying vitamin D intake is approximately twice as great as in the case of chicks.

In the absence of vitamin D, turkeys develop severe rickets even though the mineral balance of the ration is satisfactory. Poultz utilize vitamin D<sub>2</sub> and vitamin D<sub>3</sub> with about the same relative efficiency as do chicks. Limited evidence indicates that poultz can utilize dihydrotachysterol more efficiently than chicks. Turkey poultz offer advantages over chicks as test animals for vitamin D. In most cases simultaneous assays with both species indicate similar potencies for the material tested.

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### REFRACTIVE INDICES OF DEXTROSE AND INVERT SUGAR SOLUTIONS\*

By F. W. ZERBAN and JAMES MARTIN (New York Sugar Trade Laboratory, New York, N. Y.)

In a previous paper the senior author<sup>1</sup> presented a table of the refractive indices of invert sugar solutions, to the fourth decimal place. This table was based on the measurements made by de Whalley<sup>2</sup> with a sugar refractometer and expressed by him as equivalent per cent sucrose.

The new Bausch and Lomb precision refractometer makes it possible to read the refractive indices to within three units of the fifth decimal place or better throughout the entire concentration range of sugar solutions, and five-place tables are now required in order fully to utilize the increased precision. The International Table of the Refractive Indices of Sucrose Solutions (1936)<sup>3</sup> answers this requirement up to 24 per cent sucrose by weight, and Bausch and Lomb<sup>4</sup> have extended the five-place figures to 85 per cent of sucrose, by interpolation of the international four-place table.

\* Presented before the Division of Sugar Chemistry and Technology of the American Chemical Society, Cleveland meeting, April 1944.

<sup>1</sup> *This Journal*, **26**, 143 (1943).

<sup>2</sup> *Intern. Sugar J.*, **37**, 353 (1935).

<sup>3</sup> *Ibid.*, **39**, 238 (1937).

<sup>4</sup> Conversion table furnished with the instrument.

A five-place table of the refractive indices of levulose solutions has been published by Jackson and Mathews.<sup>5</sup> There are no corresponding tables for dextrose or invert sugar. The present investigation was undertaken to supply this need, and also to ascertain whether Vosburgh's rule,<sup>6</sup> as generalized by Browne,<sup>7</sup> is applicable to the refractive indices of invert sugar solutions. The measurements at 20° had been finished, and others at 25° had been partially made when conditions arising out of the war compelled interruption of the experimental work. It has therefore been decided to publish the former at this time and to resume the investigation when normal conditions return.

#### REFRACTIVE INDICES OF DEXTROSE SOLUTIONS

Following some early work on the refractive power of various sugars by Gladstone<sup>8</sup> and by Guye and König,<sup>9</sup> the first systematic determinations of the refractive indices of dextrose solutions containing up to 23 per cent of the sugar were made by Stolle,<sup>10</sup> at 17.5°. Pulvermacher<sup>11</sup> carried out measurements at 25°, the highest concentration being 25 per cent of dextrose. Data for 20, 40, and 60 per cent solutions of refined commercial dextrose at 20° were furnished by Miss Langwill.<sup>12</sup> The most recent measurements were made by Körner, Reischel, and Höppner<sup>13</sup> at 20°, through the entire concentration range up to 85 per cent. With the exception of Stolle's data at 17.5°, which extend to the fifth decimal place, all the refractive indices are given only to the fourth decimal. All the measurements were made with refractometers of the Pulfrich or Abbé type. Eventually, the fundamental values will have to be determined with the spectrometer, as was done by Schönrock with sucrose solutions. In the meantime the precision afforded by the new Bausch and Lomb refractometer should prove sufficient for practical purposes.

This instrument, provided with a G.E. sodium lamp, was used in the present work. The prism temperature was kept constant at 20° with water pumped through the jackets from a large thermostat bath. The air temperature of the work room was 20° ± 0.5. The temperature of the solutions was measured with a thermometer furnished with the instrument and calibrated by the National Bureau of Standards. The standard dextrose and the levulose used in the work on invert sugar were also secured from that institution. The solutions were prepared by weighing the sugar in glass-stoppered Pyrex Erlenmeyer flasks of about 50 ml. capacity, adding the approximate volume of distilled water to obtain the desired concentration, stoppering the flask, and gently rotating it until the sugar was

<sup>5</sup> *Bur. Standard J. Research*, **8**, 403 (1932).

<sup>6</sup> *J. Am. Chem. Soc.*, **43**, 219 (1921).

<sup>7</sup> *Louisiana Planter*, **67**, 44 (1921).

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<sup>9</sup> *Chem. Ztg.*, **19**, 1033 (1895).

<sup>10</sup> *Z. Ver. Deut. Zucker-Ind.*, **51**, 469 (1901).

<sup>11</sup> *Z. anorg. allgem. Chem.*, **113**, 141 (1920).

<sup>12</sup> *Mfg. Confectioner*, March 1941, 17.

<sup>13</sup> *Z. anal. Chem.*, **122**, 321 (1941); through *C.A.*, **36**, 5049 (1942).

completely dissolved. Careful heating in a warm water bath had to be resorted to in the preparation of the solutions of high density. The flasks were reweighed at room temperature and the concentration was calculated, allowance being made for the trace of residual moisture in the sugar. The other impurities were too small in quantity to require correction. The solutions were allowed to stand in the stoppered flask for 24 hours in order to attain rotation equilibrium. The setting of the refractometer scale was

TABLE 1.—*Refractive indices of dextrose solutions; observed data and values calculated from computed equation*

PER CENT DEXTROSE BY WEIGHT, IN AIR $p$	$n_D^{20}$ OBSERVED	$n_D^{20}$ CALCULATED FROM EQUATION (1)	DEVIATION FROM FORMULA
1.997	1.33585	1.33586	-0.00001
4.001	1.33880	1.33878	+0.00002
4.984	1.34026	1.34023	+0.00003
5.995	1.34169	1.34172	-0.00003
7.873	1.34451	1.34453	-0.00002
10.025	1.34780	1.34779	+0.00001
11.998	1.35078	1.35082	-0.00004
15.033	1.35556	1.35557	-0.00001
19.961	1.36353	1.36350	+0.00003
25.028	1.37198	1.37194	+0.00004
30.004	1.38056	1.38052	+0.00004
35.083	1.38955	1.38961	-0.00006
40.191	1.39911	1.39908	+0.00003
45.290	1.40889	1.40889	0.00000
50.246	1.41873	1.41875	-0.00002
55.237	1.42901	1.42904	-0.00003
60.452	1.44013	1.44016	-0.00003
62.652	1.44503	1.44498	+0.00005
65.746	1.45183	1.45186	-0.00003
71.036	1.46397	1.46396	+0.00001
75.615	1.47478	1.47477	+0.00001
77.022	1.47816	1.47816	0.00000
80.362	1.48632	1.48631	+0.00001

controlled by the glass test piece furnished with the instrument and was also checked with distilled water.

The experimental results are shown in Table 1. From these data the following equation was calculated by the method of averages:

$$n_D^{20}, \text{ dextrose} = 1.33299 + 0.001428288 p + 0.000004594154 p^2 + 0.00000002194551 p^3 - 0.0000000006037339 p^4 \quad (1)$$

The sum of the squares of the residuals for the 23 experiments is  $190 \times 10^{-10}$ , which is considerably smaller than that for the results of Jackson and Mathews on levulose (page 301),  $1550 \times 10^{-10}$ , comprising 32 experiments.

Table 2 gives the refractive indices of dextrose solutions, calculated



from Equation (1), in steps of 5 per cent dextrose, and also the corresponding values of Körner, Reischel, and Höppner.

It is impossible to make a direct comparison with the results of Stolle at 17.5° or with Pulvermacher's at 25° because the temperature coefficients have not been determined as yet. However, Stolle's values, read after 24 hours, are, in accordance with theory, higher throughout than those at 20° calculated from Equation (1). The differences average 0.000207, or 0.000083 for 1°C., which appears somewhat low. But Stolle found the  $n_D^{17.5}$  of water by repeated measurements to be 1.33310, which is 0.00007 lower than the accepted value. If this correction is applied to Stolle's eight measurements on dextrose solutions containing up to 23 per cent

TABLE 2.—*Refractive indices of dextrose solutions calculated from Equation (1) in steps of 5%*

PER CENT DEXTROSE BY WRIGHT, IN AIR	$n_D^{20}$ , KERRAN AND MARTIN	$n_D^{20}$ , KÖRNER, REISCHEL, AND HÖPPNER
P		
0	1.33299	1.3330
5	1.34025	1.3403
10	1.34775	1.3479
15	1.35552	1.3557
20	1.36356	1.3638
25	1.37189	1.3721
30	1.38052	1.3807
35	1.38946	1.3896
40	1.39872	1.3988
45	1.40832	1.4083
50	1.41826	1.4181
55	1.42854	1.4284
60	1.43918	1.4390
65	1.45019	1.4499
70	1.46156	1.4613
75	1.47330	1.4731
80	1.48542	1.4854
85		1.4980

of the sugar the average temperature coefficient becomes 0.000111, which seems reasonable when compared to the known values for levulose and for sucrose. Pulvermacher's results at 25° are all very much higher than those at 20° as calculated from Equation (1), although they should be considerably lower. But his  $n_D^{25}$  values for sucrose also average 0.0009 higher than those calculated for this temperature from the International Table of Refractive Indices of Sucrose Solutions. There appears to be a systematic error in the refractive indices given by Pulvermacher.

Miss Langwill's results are shown in Table 3; the concentration of water-free dextrose has been calculated from the moisture values given by her.

Miss Langwill's values throughout are lower than those calculated

TABLE 3.—*Miss Langwill's refractive indices of dextrose solutions*

PERCENT DEXTROSE BY WEIGHT, IN AIR	$n_D^{20}$ , MISS LANGWILL	$n_D^{20}$ , FROM EQUATION (1)
18.95	1.3614	1.36185
19.97	1.3632	1.36351
37.90	1.3942	1.39479
39.94	1.3981	1.39861
56.85	1.4317	1.43244
59.91	1.4388	1.43899

from Equation (1). She does not state how soon after the preparation of the solutions the refractometer readings were made. It appears that they were made soon after, since Riiber<sup>14</sup> has found that the refractive index of an  $\alpha$ -glucose solution increases upon standing.

Körner, Reischel, and Höppner give the following equation for the relation between concentration and refractive indices of dextrose solutions for sodium light at 20°:

$$p, \text{ dextrose} = -7804.48 + 14356.70 n - 8881.80 n^2 + 1878.30 n^3 \quad (2)$$

where  $p$  is per cent dextrose by weight. When this equation is solved for  $n_D^{20} = 1.33299$ , the accepted value for water,  $p = -0.04$  is obtained. Equation (2) has been converted by the writers into the usual form,  $n$  being expressed in terms of  $p$ , with this result:

$$n_D^{20}, \text{ dextrose} = 1.33306 + 0.001450156 p + 0.000004018865 p^2 + 0.00000002076620 p^3 \quad (3)$$

The refractive index of water ( $p = 0.00$ ) according to Equation (3) is 1.33306, or 0.00007 too high. This amount has been deducted from all the values calculated by this equation, and the resulting figures have been rounded off in Table 2 to the fourth decimal place, the experimental limit of the Abbé refractometer. In the range of 0–40 per cent dextrose the values of Körner, Reischel, and Höppner are up to 0.0002 higher than those of the writers, at 45 per cent they are equal, from then on they are slightly lower, and at 80 per cent there is again no difference. The discrepancies are of the same order of magnitude as those between the values found for sucrose by various investigators.<sup>15</sup>

#### REFRACTIVE INDICES OF INVERT SUGAR SOLUTIONS

The only published table of the refractive indices of invert sugar solutions is the four-place table of the senior author, based on de Whalley's data. A new series of values to the fifth decimal place has been determined in the same manner as described for dextrose, upon mixtures of equal quantities of dextrose and levulose, taking into consideration the small amount of moisture in the dextrose. The levulose was dried to constant weight in vacuo at 50°C. The readings were made 24 hours after the

<sup>14</sup> Ber., 56B, 2185 (1923); 57B, 1599 (1924); 58B, 737 (1925).

<sup>15</sup> BROWNE AND ZERBAN, "Physical and Chemical Methods of Sugar Analysis," 3rd ed., 1941, p. 101, Table XXVI.

preparation of the solutions. The experimental results are shown in Table 4.

The following equation has been computed from the observed values:

$$n_D^{20}, \text{ invert sugar} = 1.33299 + 0.001411210 p + 0.000005198919 p^2 + 0.00000001568555 p^3 - 0.0000000003776638 p^4 \quad (4)$$

The sum of the squares of the residuals for the 23 experiments is  $233 \times 10^{-10}$ , a little higher than that found for the dextrose equation (1).

Balch<sup>16</sup> has reported that a solution containing 77.79 per cent invert sugar shows the same refractive index as a 76.12 per cent sucrose solution,

TABLE 4.—*Refractive indices of invert sugar solutions; observed data and values calculated from computed equation*

PER CENT INVERT SUGAR BY WEIGHT, IN AIR <i>p</i>	$n_D^{20}$ OBSERVED	$n_D^{20}$ CALCULATED FROM EQUATION (4)	DEVIATION FROM FORMULA
2.002	1.33583	1.33584	-0.00001
3.941	1.33863	1.33863	0.00000
4.993	1.34018	1.34017	+0.00001
6.002	1.34163	1.34165	-0.00002
8.002	1.34462	1.34462	0.00000
10.001	1.34766	1.34764	+0.00002
14.987	1.35536	1.35536	0.00000
19.998	1.36343	1.36341	+0.00002
25.080	1.37196	1.37189	+0.00007
30.097	1.38051	1.38057	-0.00006
35.203	1.38971	1.38974	-0.00003
40.050	1.39875	1.39876	-0.00001
44.985	1.40827	1.40827	0.00000
49.981	1.41823	1.41823	0.00000
54.929	1.42841	1.42845	-0.00004
59.935	1.43919	1.43914	+0.00005
62.444	1.44459	1.44463	-0.00004
64.994	1.45033	1.45030	+0.00003
67.396	1.45572	1.45574	-0.00002
69.954	1.46162	1.46162	0.00000
72.465	1.46750	1.46748	+0.00002
74.936	1.47329	1.47334	-0.00005
79.853	1.48533	1.48528	+0.00005

1.4802 at 20°. The value calculated from Equation (4) is 1.48023, a very close check.

The refractive indices of invert sugar solutions in steps of 5 per cent, calculated from Equation (4), are given in Table 5. The values based on de Whalley's data are shown in comparison, and also those calculated by the Vosburgh-Browne rule from the refractive indices of dextrose solutions (Table 2) and of levulose solutions. The latter were not taken from the table of Jackson and Mathews, but were calculated from an unpublished equation of the same form as Equation (4), computed by J. B. Saunders,

<sup>16</sup> *This Journal*, 15, 176 (1932).

of the National Bureau of Standards, from the experimental values of Jackson and Mathews and fitting their data better than the original three equations covering different portions of the concentration range.

Above the 5 per cent concentration, de Whalley's values are from 0.0001 to 0.0003 higher than those found by the writers, but they check with them at 65 per cent invert sugar. Aside from experimental errors, these discrepancies may be due partly to incomplete mutarotation equilibrium when the solutions were read by de Whalley, and partly to the ash content

TABLE 5.—*Refractive indices of invert sugar solutions, calculated from Equation (4), in steps of 5%*

PER CENT INVERT SUGAR BY WEIGHT, IN AIR	$n_D^{20}$ , ZERBAN AND MARTIN	$n_D^{20}$ , DE WHALLEY- ZERBAN	0.5 $n_D^{20}$ DEXTROSE PLUS 0.5 $n_D^{20}$ LEVULOSE
0	1.33299	1.3330	1.33299
5	1.34018	1.3402	1.34022
10	1.34764	1.3478	1.34769
15	1.35538	1.3556	1.35543
20	1.36341	1.3637	1.35346
25	1.37175	1.3720	1.37178
30	1.38040	1.3807	1.38042
35	1.38937	1.3896	1.38937
40	1.39866	1.3988	1.39865
45	1.40830	1.4085	1.40827
50	1.41827	1.4186	1.41823
55	1.42860	1.4289	1.42853
60	1.43928	1.4395	1.43918
65	1.45032	1.4503	1.45018
70	1.46172		1.46153
75	1.47350		1.47323
80	1.48564		1.48527

in the invert sugar used by him, which amounted to 0.025 per cent. According to Riiber<sup>14</sup> the  $n_D^{20}$  of a 10 per cent solution of  $\beta$ -fructose decreases 0.00029 upon conversion into  $\alpha\rightleftharpoons\beta$ -fructose, but that of an  $\alpha$ -glucose solution increases only 0.00009 upon conversion into  $\alpha\rightleftharpoons\beta$ -glucose.

The writers' values calculated from Equation (4) for invert sugar check fairly closely with the arithmetical average of the refractive indices of dextrose and levulose, up to an invert sugar concentration of 50 per cent. At the lowest concentrations, up to 20 per cent, they are 0.00004–0.00005 lower. Then the difference decreases and becomes 0 for 35 per cent invert sugar. Above that point the difference is in the opposite direction, and gradually rises to 0.00007 at 55 per cent invert sugar, and finally to 0.00037 at 80 per cent. The deviation of the values calculated by the Vosburgh-Browne rule from those based on the experimental data are of about the same order of magnitude as those found by Jackson and McDonald<sup>17</sup> for the saccharimetric values of invert sugar. At a concentration

<sup>17</sup> *This Journal*, 25, 875 (1942).

of around 20 per cent of invert sugar by weight the application of the rule to the refractometric or saccharimetric values for dextrose and levulose causes an error of a little under 0.1 per cent of invert sugar, or 0.5 per cent of the total invert sugar present. At a concentration of 80 per cent of invert sugar, determined with the refractometer, the error amounts to 0.15 per cent invert sugar, or about 0.2 per cent of the total. This confirms the conclusion of Jackson and McDonald that the Vosburgh rule is a close approximation. Vosburgh himself found that it holds well for the rotation of mixtures of glucose and sucrose, and of glucose and fructose, but only approximately in the case of sucrose and fructose.

#### SUMMARY

The refractive indices of dextrose and invert sugar solutions for sodium light at 20° have been measured with the Bausch and Lomb precision refractometer, and equations for the relation between the refractive index and the percentage of sugar by weight in air have been computed. Tables of the experimental and computed values, to the fifth decimal place of  $n$ , are presented, and these values are compared with previous data in the literature. It has been found that the Vosburgh-Browne rule about the physical properties of mixed sugar solutions applies closely to the refractive indices of equimolecular mixtures of dextrose and levulose.

### METHODS FOR DIRECT COUNT OF MICROORGANISMS IN CITRUS PRODUCTS

By J. W. STEVENS and T. C. MANCHESTER (Research Department,  
California Fruit Growers Exchange, Ontario, Calif.)

Plate counts are a measure of live microorganisms only, and do not adequately indicate the quality of fruit used or the sanitary precautions employed in the manufacture of citrus products. Because some form of heat or chemical treatment is generally used to insure practical sterility, products containing enormous numbers of dead microorganisms may show negative plate counts. The presence of these dead microorganisms may be due to the use of poor quality fruit or to inadequate sanitation in manufacture, or to both.

Some method of count by direct microscopic examination is considered necessary to complete the bacteriological evaluation of these products. The methods of Breed<sup>1,2</sup> and Howard<sup>3,4</sup> are available, but both require adaptation for use with citrus products. The method developed and described herein is based on the Breed procedure.

<sup>1</sup> *Centra. Bakt.*, Abt. II, 30, 337-40 (1911).

<sup>2</sup> Breed and Brew, N. Y. Agr. Expt. Sta. Tech. Bull. 49 (1916).

<sup>3</sup> U. S. Dept. Agr. Bur. Chem. Circ. 68 (1911).

<sup>4</sup> Howard and Stephenson, U. S. Dept. Agr. Bull. 581 (1917).

In the development of this method it was found that satisfactory films could not be prepared directly from the juice. Separation of the soluble from the insoluble solids by centrifuging was of value but this eliminated only one of the difficulties. It was evident that the juice contained an insufficient amount of materials which serve as fixing agents in the heat fixation of the dried films. Also, difficulty was encountered with clumping of insoluble solids in the film and with collection of a relatively large portion of the bacterial cells in the edges of the film. These difficulties were finally overcome by adding a small amount of gelatin to the juice and then fixing the gelatin-containing film by treatment with formaldehyde solution. This procedure allows the films to be prepared without centrifuging, thus eliminating that time-consuming step. However, centrifuging is of possible value when examining low-count samples containing little suspended material and/or much added sugar. Successive angle (using Baier's adaptor<sup>6</sup>) and horizontal centrifuging was found somewhat more effective than either alone.

#### A. METHOD FOR CITRUS JUICE PRODUCTS

This method is similar in many respects to the Breed procedure as described in "Standard Methods for the Examination of Dairy Products"<sup>6</sup> and for this reason many of the details given in the latter method are applicable to both procedures.

*I. Preparation of Films.*—One ml. of the juice product is transferred with a clean 1 ml. pipet to a clean 10 or 25 ml. graduated cylinder. If the product is too viscous to be handled with a pipet, the portion is weighed into the cylinder, a weight equivalent to 1 ml. being used. From 1 to 11 ml. of redistilled water (free from microorganisms) is then added, depending upon the amount of dilution desired. To the resulting solution is added an equal volume of a freshly prepared 0.5 per cent solution of gelatin,\* made with redistilled water. The cylinder is closed with a clean stopper and the contents are thoroughly mixed by inverting the cylinder at least 25 times.

Two 0.01 ml. portions of the solution are transferred at once, with a 0.01 ml. pipet, before separation or settling of insoluble solids occurs, to appropriate squares on the slide. These squares, with 1 cm. sides, may be etched on the slide or a guide may be used to indicate the area to be covered. The portions are quickly and evenly spread over the outlined surfaces with a platinum needle. The films are allowed to air dry at room temperature, heated gently by passing three times through a small flame, fixed by flooding with formalin solution† (35–40 per cent formaldehyde), and allowed to stand for 1.5 minutes. The excess formalin solution is

<sup>6</sup> *Ind. Eng. Chem., Anal. Ed.*, 16, 193 (1944).

<sup>6</sup> Pub'd by American Public Health Assoc. and Assoc. of Official Agricultural Chemists, 8th ed., pp. 40–57 (1941).

\* The gelatin used should be known by test to be practically free from microorganisms. The two lots of Difco standardised gelatin used in this work were satisfactory.

† The use of glyoxal has been proposed as an "insolubilizing agent" for gelatin and other protein materials, and was used by Wicks and Sontseff (7) to harden animal tissues.

poured off, and the films are allowed to stand at room temperature until partially dry. They are then washed gently with 35 per cent alcohol and then with distilled water; finally they are air dried at room temperature.

*II. Staining of Films.*—The air-dried films are flooded with alcoholic methylene blue solution, and gentle heat is applied occasionally for a period of 2 minutes. The excess stain solution is then poured off and the films are thoroughly washed by careful dipping in two or more successive portions of distilled water. After air drying at room temperature the films are ready to be examined.

Alcoholic methylene blue solution is prepared by adding an excess of the dye to a mixture consisting of 1 part 95 per cent alcohol and 1.5 parts of redistilled water, by volume. It is allowed to stand overnight or longer and then filtered through Whatman's No. 1 filter paper. This solution is very stable.

*III. Microscopic Examination of Films.*—Both films of the sample are examined with a compound microscope equipped with oil immersion objective (about 95 $\times$ ), 8 or 10 $\times$  ocular, and a Breed and Brew ocular micrometer disc. The microscope is standardized as described in the Breed method.<sup>7</sup>

The number of fields to be counted on each film depends on the number of microorganisms per field and the precision required. If the first few fields show less than 50 microorganisms each, of any one class (yeast, mold spores, mold mycelia, or bacteria), it will be necessary to count this class in 50 fields (25 fields on each of the 2 films) for a probable error of about 10 per cent. When the number per field is greater than 50, it is necessary to count the particular class of microorganism in a total of only 25 fields to maintain roughly the same probable error. A count of more than 100 fields (divided between the 2 films) appears to be justified in but few instances, regardless of how few microorganisms are present per field.

In making the actual counts it is necessary that the fields examined be so distributed that uniform coverage of each film is obtained. The yeast, mold spores, mold mycelia, and bacteria are counted, and recorded separately for each field. Bacteria, being smaller individuals and often greater in number, are counted only within the inner circle of the eyepiece micrometer disc where definition is best. When the required number of fields has been counted on the first film the figures are totaled for each class. These sums are divided by the number of fields examined to obtain the mean count per field for each class of microorganism.

The same procedure is followed with respect to the second film, and the means are averaged. From this value the number of microorganisms per ml. may be calculated by substituting the known values in the following formula:

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<sup>7</sup> *Science*, 98, No. 2539, 204 (1943).

Number of microorganisms per ml. = (fields per cm.<sup>2</sup> × 100) × average count per field × dilution.

## B. METHOD FOR POWDERED PECTIN

Frequently it is desirable to use the same sample of pectin for both plate count and direct microscopic count. It is then necessary that the apparatus used in handling both the powdered pectin and the pectin sol be sterile as well as physically clean.

*I. Preparation of Pectin Sol.*—In preparing a 2 per cent sol, which is the most convenient strength, 2 grams of pectin are weighed out rapidly on the analytical balance in a sterilized beaker and then transferred quickly to a sterilized 250 ml., wide-mouthed, glass-stoppered bottle. The bottle is tilted, and then tapped on the table top to cause the pectin to collect on one side of the bottom. The bottle is next slightly tilted in the opposite direction and 7 ml. of 60° Brix simple sirup is added from a pipet so as not to come in contact with the pectin. When this addition is complete, the sirup and pectin are mixed and stirred rapidly with a sterilized glass rod until all lumps of pectin have been broken up and a smooth suspension formed. Before too much hydration of the pectin can occur a 92 ml. portion of sterilized, redistilled water is added all at once, the stopper is placed in the bottle, and the bottle is agitated until dispersion of the pectin is complete.

The 60° Brix sirup must be practically free from microorganisms. It is prepared by dissolving 772 grams of sucrose in 515 grams of redistilled water and placed in 2 ounce bottles (allow ample head space), and the bottles are closed with cotton stoppers; it is then sterilized for 1 hour at 15 pounds, and after the cotton stoppers have been replaced aseptically with paraffined corks, it is stored until needed.

The pectin may also be dispersed by the use of sand. The 2 gram portion of pectin is mixed with about 12 grams of clean 40–60-mesh sand which has been sterilized and dried. The mixture is poured into 99 ml. of sterilized, redistilled water while stirring with a sterilized glass rod; stirring is continued until dispersion is complete.

*II. Direct Total Count.*—The 2 per cent pectin sol is mixed with 0.25 per cent gelatin solution and redistilled water in the proportions of 1 part pectin sol, 2 parts gelatin solution, and 1 part redistilled water, by volume. This gives a final solution containing 0.5 per cent pectin and 0.125 per cent gelatin, which is used for preparing stained films.

From this point the procedure is identical with that described under A.

*III. Direct Spore Count.*—The procedure followed in the above direct count does not show yeast and bacterial spores because of the difficulty of staining these bodies.

Neisser's spore-staining procedure as described by Tanner<sup>8</sup> was found to be satisfactory for use with citrus juices and pectin. The films are

<sup>8</sup> "Bacteriology and Mycology of Food," 1st ed., p. 85. John Wiley & Sons, Inc., New York (1919).



stained with aniline water-fuchsin, partially decolorized with alcoholic hydrochloric acid, and finally counterstained with methylene blue (as described under A). The spores are stained red and the vegetative cells blue, thus permitting spore counts, in addition to the usual total counts, with the same film.

### C. DISCUSSION

The extent of dilution necessary in the examination of citrus juice products is determined (a) by the insoluble solids (pulp, etc.) content of the

TABLE 1.—*Counts obtained with different citrus juice products*  
(Data obtained during development of method in 1937.)

KIND OF JUICE	VOLUME CONCENTRATION RATIO AVERAGE (APPROX.)	NUMBER OF SAMPLES EXAMINED	RANGE OF COUNT	MICROORGANISMS (IN THOUSANDS) PER ML.				
				YEASTS	MOLD SPORES	MOLD MYCELIA	BACTERIA	TOTAL
<i>Concentrated orange.</i> Juice expressed from whole orange with rolls, reeled, and then passed through centrifugal separator, vacuum concentrated (I)	6.9:1	4	High Low Average	20,100 4,000 12,000	24,800 5,000 16,900	<70 210 —	279,300 34,000 164,600	324,300 55,700 193,600
<i>Concentrated orange.</i> Juice extracted by hand reaming, then put through finisher with fine screen, vacuum concentrated (II)	4.0:1	5	High Low Average	4,200 1,000 2,100	3,600 490 1,100	<70 <35 —	34,500 2,200 10,100	42,300 3,700 13,400
<i>Orange pulp juice, coarse.</i> Whole unpeeled fruit macerated and then put through finisher with coarse screen (III)	none	2	High Low Average	1,800 900 1,300	280 250 260	<35 <35 <35	15,700 13,200 14,500	17,600 3,900 16,000
<i>Orange pulp juice, fine.</i> Whole unpeeled fruit macerated and then put through finisher with fine screen (IV)	none	2	High Low Average	1,800 900 1,300	770 490 630	<35 <35 <35	8,600 6,600 7,600	10,000 9,100 9,600
<i>Canned orange juice.</i> Juice extracted by hand reaming, then put through finisher with fine screen	none	2	High Low Average	900 500 700	1,700 1,500 1,600	<32 <32 <32	2,000 1,400 1,700	4,200 3,900 4,000
<i>Mixture.</i> Containing juices (I) and (II), citric acid, sugar, and flavoring oils	1.7:1	2	High Low Average	900 900 900	1,100 500 800	32 <32 —	8,300 2,300 5,300	10,200 3,700 7,000
<i>Mixture.</i> Containing juices (I), (II), and (IV), citric acid, sugar, and flavoring oils	1.7:1	2	High Low Average	3,600 1,100 2,400	2,600 2,500 2,600	<64 <32 —	14,700 6,800 10,800	20,900 10,400 15,700
<i>Mixture.</i> Containing orange juice, citric acid, sugar, and flavoring oils but varying in composition*	2.5:1	5	High Low Average	3,400 125 1,100	1,100 125 430	66 <16 —	4,200 470 2,300	8,700 900 3,700
<i>Mixture.</i> Containing orange juice, citric acid, sugar, and flavoring oils but varying in composition*	4.2:1	7	High Low Average	4,000 159 1,300	7,200 350 2,700	32 <16 —	5,200 1,100 2,900	12,200 1,800 7,000
<i>Concentrated lemon.</i> Juice expressed from whole unpeeled fruit with rolls, reeled, then passed through centrifugal separators (V), vacuum concentrated	3.2:1	2	High Low Average	<32 32 <32	1,100 500 800	<32 <32 <32	3,400 2,700 3,100	3,900 3,800 3,800
<i>Concentrated lemon.</i> Juice (V), vacuum concentrated	5.8:1	5	High Low Average	<32 <32 <32	640 220 410	<32 <32 <32	2,300 1,100 1,600	2,800 1,400 2,000
<i>Canned lemon juice.</i> Juice (V)	none	2	High Low Average	<32 <32 <32	320 159 240	<32 <32 <32	1,900 700 1,800	2,100 1,100 1,600

\* Some contained all of indicated ingredients, others only part of them.

TABLE 2.—*Results of direct counts of pectin samples*  
(Data obtained from samples collected in 1941.)

SAMPLE NUMBER	MICROORGANISMS (IN THOUSANDS) PER GRAM OF SAMPLE				
	YEASTS	MOLD SPORES	BACTERIAL AND YEAST SPORES	BACTERIA	TOTAL
1	<650	<650	—	4,500	4,500
2	<650	<650	—	1,900	1,900
3	2,500	<650	—	1,900	4,400
4	43,900	<650	—	15,900	59,800
5	17,200	<650	—	14,000	31,200
5	17,800	650	3,200	16,500	38,200

sample, which in turn is largely determined by the type of juice and by the concentration ratio; and (b) by the number of microorganisms present. The greater the amount of insoluble solids the higher the dilution required to prevent the final dried film from being too dense when observed under the microscope. In general the dilution is kept as low as (a) will permit, except occasionally where (b) is so high as to become the controlling factor. Usually a dilution which will give a solution of about 0.5 natural juice strength will be found satisfactory but sometimes dilution to as low as 0.1 natural strength is required.

The strength of pectin sol that can be used with the method is limited by the density of the stained film with respect to the passage of light. Because of this, sols of more than about 0.5 per cent, at final dilution, are not generally satisfactory.

The results obtained with the method on a number of citrus juice products are shown in Table 1. These samples represent several types of products, made by several different manufacturers. The types of juice included were selected to represent a wide range in amount as well as particle size of insoluble solids.

Data pertaining to the total microorganism content of 5 samples of commercial powdered pectin are shown in Table 2.

No attempt is made to evaluate the data presented in Tables 1 and 2 from a sanitary standpoint because of the complicated nature of the problem. The data appear to be of sufficient interest to warrant further consideration of the method as a tool in the bacteriological study of citrus products. Also, the method presented would appear to be readily adaptable to fruit products other than citrus.

#### ACKNOWLEDGMENT

The authors are grateful to W. E. Baier for suggestions and assistance, and to G. H. Joseph for suggesting the use of formalin in the fixation of the gelatin-containing films.

## A STUDY OF THE TOXICITY FOR CATS OF THOSE NIGHTSHADE BERRIES OCCASIONALLY FOUND IN CANNED PEAS

By WM. T. McCLOSKEY and WM. V. EISENBERG\* (U. S. Food and Drug  
Administration, Federal Security Agency, Washington, D. C.)

Examinations of canned peas have revealed that cans occasionally have contained as a contaminant the immature green berries of the black nightshade, *Solanum nigrum* L., and the yellow nightshade, *S. villosum* Mill. Both species occur as weeds in the pea-growing areas of the Northwest and find their way into the canneries during the harvest. The two species are very closely related and differ essentially in the greater amount of pubescence in *villosum* and the color of the mature fruit, which is black in *nigrum* and yellow in *villosum*. The size and appearance of the green berries from these plants are such that they could easily be mistaken by the ordinary consumer for canned peas. Although the immature berries have an objectionable flavor, which of itself makes them undesirable in canned peas, the long history of reports indicating the poisonous character of the black nightshade, *S. nigrum*, made it important that an investigation be conducted to determine whether or not these contaminants of canned peas are actually toxic.

In the present study authentic specimens of *S. nigrum* L. and *S. villosum* Mill.† secured from various parts of the country were fed to cats. Thirty-five collections of plants of *S. nigrum* with attached berries were gathered from different localities in the states of California, Delaware, Georgia, Illinois, Louisiana, Maryland, Minnesota, Missouri, New York, Pennsylvania, Washington, and the District of Columbia. From these, 57 feedings with green berries and 15 with ripe black berries were conducted. Eight collections of *S. villosum* from different localities in the states of California, Oregon, Utah, and Washington allowed 16 feedings with the green berries from these plants.

The berries from the collected specimens were fed to the animals in portions of 20 berries of each type from a single collection. This number was selected because it exceeded the amounts found in the No. 2 size can of peas and represented a portion far in excess of what a person might consume of this accidental contaminant in canned peas. The 20 berries were weighed and cut into small pieces. They were then incorporated into 100 grams of ground beef and fed to apparently healthy cats. The other members of the cat colony were used as controls. The controls received the ground meat ration without the addition of berries. The period of observation was for 48 hours after the berries were fed. No symptoms of any kind were noted. The feeding data are summarized and tabulated below.

\* Joint contribution from Division of Pharmacology and Microanalytical Division.

† The authors wish to express their thanks to S. F. Blake of the Bureau of Plant Industry, U.S.D.A., for checking the identification of these specimens.

*Summary of data on nightshade berries fed to cats*

SPECIMEN	FEEDINGS	AVERAGE AND RANGE OF WEIGHT OF 20 BERRIES	AVERAGE AND RANGE OF WEIGHT OF CATS	GRAMS OF BERRIES PER KG. OF CAT
		grams	grams	
<i>S. nigrum</i> green berries	57	3.5 (1.3-8.6)	2754 (1950-4475)	1.3
<i>S. nigrum</i> ripe berries	15	4.8 (2.4-10.0)	2887 (2265-3325)	1.7
<i>S. villosum</i> green berries	16	4.0 (3.2-5.5)	2996 (2435-3425)	1.3

The results of this investigation clearly show that nightshade berries of the species *S. nigrum* L. and *S. villosum* Mill., currently found in occasional commercial samples of canned peas, are not toxic to cats and probably do not represent a serious problem from the standpoint of toxicity to the consumer. Other animal species will be tested as time permits. The presence of these berries, however, is an undesirable adulteration because of their objectionable flavor, and the efforts of the canner should be directed to their removal from canned peas.

## VOLUMETRIC DETERMINATION OF ALKANOLAMINES IN EMULSIONS

By J. H. JONES (Cosmetic Division, Food and Drug Administration,  
Federal Security Agency, Washington, D. C.)

The alkanolamines combine with the higher fatty acids to form soaps that are useful as emulsifiers, and a number of such compounds have been proposed for use in the preparation of cosmetic creams and lotions. Among these are the mono-, di- and triethanolamines; tri-isopropanolamine and 2-amino-2-methyl-1,3-propanediol ("amino glycol"). Still other compounds of this type are available and may be found in cosmetics.

Gravimetric methods for mono-, di-, and triethanolamine<sup>1,2,3</sup> have been published but apparently the quantitative determination of other amines of this type in emulsions has not been investigated. Usually the amine content of emulsions has been estimated from a nitrogen determination.<sup>4</sup>

In aqueous solution these amines can be accurately titrated. However, the general procedures for the isolation of organic amines can not be used here since most alkanolamines are neither extractable with organic solvents nor volatile with steam. Experiments have shown, however, that they may be separated from the interfering substances commonly present in cosmetic emulsions by the following procedure: The amine is extracted from the emulsion with hydrochloric or hydrobromic acid, excess acid is

<sup>1</sup> Shupe, I. S., *This Journal*, 24, 754, (1941).

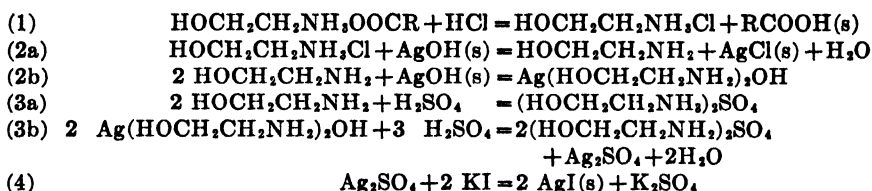
<sup>2</sup> Fleck, H. R., *Analyst*, 60, 77 (1935).

<sup>3</sup> Eastland, C. J., Evers, N., and West, T. F., *Analyst*, 62, 261 (1937).

<sup>4</sup> Biffen, F. M., and Snell, F. D., *Ind. Eng. Chem., Anal. Ed.*, 15, 517 (1943).

removed by evaporation, and the amine is liberated by treatment with silver oxide. After separation from the precipitated silver halide and excess silver oxide by filtration, the liberated amine is titrated with standard acid. The necessary correction for dissolved silver oxide is easily determined by titration with potassium iodide solution and starch-iodine indicator.<sup>5</sup>

For monoethanolamine the reactions involved may be represented by the equations:



Similar equations may be written for other amines.

All of these reactions except that represented by Equation (2b) are quantitative. The equilibrium conditions in reaction (2b) are determined by the amine present and the concentration of the various reactants. Examination of Equations (3a) and (3b) shows that the total acid titration exceeds that required by the amine alone by an amount equal to the number of equivalents of dissolved silver oxide. The dissolved silver may be readily determined by the titration represented by Equation (4). Thus, the difference between the two titrations gives the acid needed to neutralize the amine alone.

#### PROPOSED METHODS

##### REAGENTS

(a) *Silver oxide suspension*.—Dissolve 10 grams of  $\text{AgNO}_3$  in approximately 100 ml. of water and add a slight excess of  $\text{NaOH}$ . Wash the precipitated  $\text{Ag}_2\text{O}$  with water by decantation until free from  $\text{NaOH}$ . Transfer to a glass-stoppered bottle and add enough water to make the total volume approximately 200 ml. Shake thoroughly immediately before use.

(b) *Iodine-sulfuric acid solution (free from iodide)*.—Shake 5–10 grams of c.p. I with 50–75 ml. of approximately 1  $N \text{ H}_2\text{SO}_4$  in a glass-stoppered flask. Decant and discard the solution. Repeat the process until 5 ml. of the supernatant liquid gives no color with 50 ml. of water containing 2 ml. of 0.5% starch solution.

(c) *Starch indicator*.—A 0.5% solution of soluble starch in water.

(d) *Methyl red indicator*.—A 0.5% solution of methyl red in alcohol.

(e) *Standard sulfuric acid solution*.—0.02  $N$ . Dilute 200 ml. of accurately standardized 0.1  $N \text{ H}_2\text{SO}_4$  to exactly 1 liter with distilled water.

(f) *Standard iodide solution*.—0.02  $N$ . Dry c.p. KI for several hours at  $135^\circ\text{C}$ . Dissolve 3.320 grams of the dried salt in exactly 1 liter of distilled water.

##### PROCEDURE

*Extraction of the amine*.—Place a weighed sample of 1–4 grams in a 200 ml. beaker, add 10–20 ml. of acetone, and heat on the steam bath until the material dis-

<sup>5</sup> Kolthoff and Furman, "Volumetric Analysis" Vol. II, p. 233. John Wiley and Sons, New York (1929).

solves or a fine suspension is obtained. Add 2–3 ml. of HCl (or HBr) and stir thoroughly. Slowly add 15–20 ml. of water and continue to heat until the acetone is volatilized. Remove from the steam bath and cool until the oils solidify. (Add stearic acid to raise the solidification point of liquid oils.) Loosen the solid oil layer from the sides of the beaker and decant the aqueous layer into a 100 ml. volumetric flask. Rinse the beaker and oil cake with approximately 10 ml. of water. To the oil remaining in the beaker, add 10–15 ml. of water and 2–3 drops of acid, heat to boiling, and stir thoroughly. Cool, and decant as before. Repeat the extraction, cool, decant, and finally wash the beaker and oil with several small portions of water. Bring the combined water extracts to room temperature, dilute to the mark with water, and mix thoroughly.

*Isolation and determination of the amine.*—Place an aliquot of the acid extract containing 0.05–0.20 milliequivalents of base in a small beaker, concentrate to 2–5 ml., place on the steam bath, and evaporate to dryness. Dissolve the residue in approximately 5 ml. of water and add 1 drop of methyl red indicator. Add an excess of the silver oxide suspension (5 ml. is usually sufficient), stir thoroughly, wash down the sides of the beaker with a little water, and let stand for 5–10 minutes. Filter through a quantitative filter paper (the filtrate should be clear or only very faintly cloudy) into a 125 ml. titration flask and wash the beaker and filter with three 5 ml. portions of water. Titrate the filtrate with 0.02 *N* H<sub>2</sub>SO<sub>4</sub> to a permanent methyl red end point. (A sharp change from the intermediate to the acid color of the indicator occurs at the end point.)

Add 2 ml. of the 0.5% starch solution and 5 ml. of the iodine-sulfuric acid solution and titrate dropwise with 0.02 *N* KI to a permanent blue end point. Subtract from this titration the amount of 0.02 *N* KI required to give a deep blue color to a mixture of 50 ml. of water, 2 ml. of the starch solution, and 5 ml. of the iodine-sulfuric acid solution. (This blank should be less than 0.05 ml. of 0.02 *N* KI.)

Subtract the corrected KI titration from the H<sub>2</sub>SO<sub>4</sub> titration to obtain the acid equivalent to the amine alone.

1 ml. of 0.02 *N* H<sub>2</sub>SO<sub>4</sub> = 1.222 mg. of monoethanolamine  
= 2.103 mg. of diethanolamine  
= 2.984 mg. of triethanolamine  
= 3.825 mg. of tri-isopropanolamine  
= 2.103 mg. of "amino glycol"

#### COMMENTS

Methyl red appears to be the most suitable indicator for the titration of the alkanolamines. Sharp, reproducible end points are obtained in both macro and semi-micro titrations. Shupe<sup>6</sup> found that in the titration of morpholine, the basic properties of which are similar to those of the alkanolamines, the methyl red end point and equivalent point were identical.

No samples of pure alkanolamines were available to check the accuracy of the titrations. A commercial sample of 2-amino-2-methyl-1,3-propanediol was recrystallized from acetone and dried for several days over calcium chloride. The nitrogen content and macro titrations with either methyl red or methyl orange as indicator pointed to a purity of 97.5–98.0 per cent. Figure 1 shows the significant portion of the titration curve of a semi-micro sample of this product and that the equivalent point and the

<sup>6</sup> Shupe, I. S., *This Journal*, 23, 824 (1940).

methyl red end point (acid color) coincide. Other alkanolamines would undoubtedly give similar titration curves since 0.2 to 0.002 *M* solutions

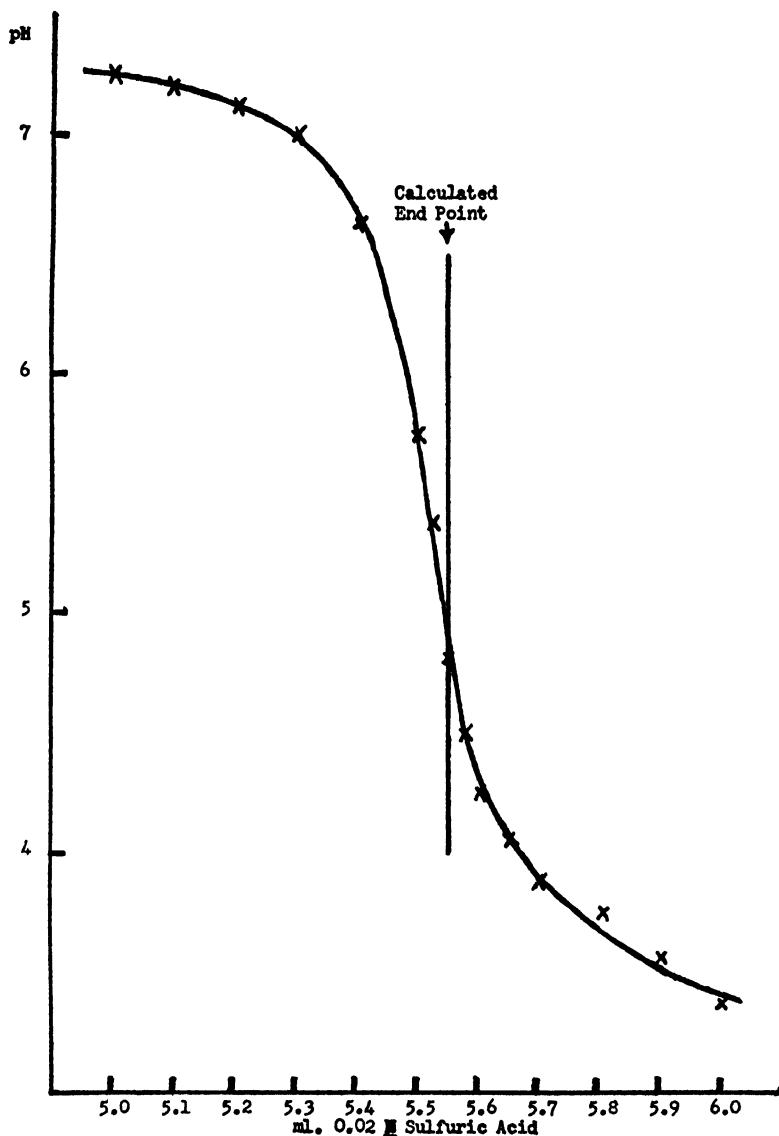


FIG. 1.—TITRATION CURVE OF 2-AMINO-2-METHYL-1,3-PROPANEDIOL (11.90 MG. IN 25 ML. OF WATER).

of the purified hydrochlorides of monoethanolamine, triethanolamine, triisopropanolamine, and 2-amino-2-methyl-1-propanol were all neutral or just acid to methyl red.

The titration of silver with iodide and starch-iodine indicator in acid solution gives results accurate to 0.5 per cent in solutions as dilute as 0.001 *N*. The alkanolamines do not interfere in the determination of silver by this method.

The proposed procedure for the determination of alkanolamine in alkanolamine-silver oxide mixtures was tested as follows: For alkanolamine-silver oxide solutions an aliquot of an approximately 0.02 *N* solution of the amine was treated with excess silver oxide. After 10–15 minutes the solution was filtered directly into a titration flask, and the beaker and filter were thoroughly washed with several small portions of distilled water. The alkanolamine content of this solution was then determined by the proposed procedure. Typical results are shown in Table 1. In each

TABLE 1.—*Titration of alkanolamine-silver oxide mixtures*

AMINE	ALIQOT	0.02 <i>N</i> H <sub>2</sub> SO <sub>4</sub> USED	0.02 <i>N</i> KI USED	NET TITRATION
	ml.	ml.	ml.	ml.
Diethanolamine	10.00	7.55	—	7.55*
	10.00	7.58	—	7.58*
	10.00	8.55	0.97	7.58
	5.00	4.60	0.82	3.78
Monoethanolamine	10.00	8.15	—	8.15*
	10.00	11.80	3.65	8.15
	5.00	6.20	2.15	4.05

\* Direct titration, no silver oxide present.

case, the net titration is substantially the same as that obtained in the direct titration of the amine solution.

In the determination of the amine, extracted from emulsions, an equivalent or excess amount of chloride is present. This chloride is converted to silver chloride in the treatment with silver oxide. If any silver chloride is dissolved as the result of complex formation it will be slowly converted to silver iodide in the iodide titration. This causes the starch-iodine end point to fade, and if the titration is continued to a permanent blue color, the iodide titration will not be equivalent to the dissolved silver hydroxide. Silver chloride is slightly soluble in dilute aqueous solutions of amines; however, the presence of excess silver oxide, which is much more soluble in amine solutions, should greatly depress the solubility of the chloride. No evidence of the solution of titratable amounts of silver chloride could be found when the proposed procedure was used. If solution of silver chloride should occur, the use of hydrobromic acid would eliminate this source of error, since silver bromide is much less soluble in amine solutions than the chloride. Substitution of hydrobromic for hydrochloric acid did not affect the recovery of any of the alkanolamines investigated. With either



acid, if the filtrate is clear, a sharp permanent end point is obtained and the results of the analysis are satisfactory.

Any uncoagulated silver chloride which passes through the filter will have the same effect as dissolved silver chloride. It is not advisable to use heat at this stage in order to coagulate the silver chloride precipitate. This usually causes some reduction of silver compounds to metallic silver, and furthermore ammonia or volatile amines, if present, may be partially expelled by heating. A cloudy filtrate is rarely encountered in the analysis of alkanolamines by the proposed procedure, but should this occur, a re-filtration through the same filter usually eliminates the cloudiness. Also, if subsequent aliquots are filtered through the paper used for the first

TABLE 2.—*Analysis of purified compounds*

COMPOUND	SAMPLE SIZE	0.02 N H <sub>2</sub> SO <sub>4</sub> USED	0.02 N KI USED	AMINE	
				FOUND	CALCULATED
	mg.	ml.	ml.	mg.	mg.
Monoethanolamine hydrochloride	11.3	7.60	1.80	7.09	7.08
	11.3	7.45	1.65	7.09	7.08
	11.3	7.42	1.60	7.11	7.08
Triethanolamine hydrochloride	20.0	5.87	0.45	16.2	16.1
	20.0	5.83	0.45	16.1	16.1
	40.0	11.10	0.45	31.8	32.1
Tri-isopropanolamine hydrochloride	23.0	5.48	0.45	19.2	19.3
	23.0	5.60	0.53	19.4	19.3
2-Amino-2-methyl-1- propanol hydro- chloride	8.00	4.33	1.13	5.70	5.68
	16.0	8.62	2.20	11.4	11.4
	16.0	8.40	2.00	11.4	11.4

aliquot, the filtrate is practically always clear. When hydrochloric acid is used for the extraction of emulsions which contain ammonia, it is sometimes difficult to secure a clear filtrate even though re-filtration is employed. For the extraction of such emulsions the use of hydrobromic acid is indicated.

A number of alkanolamine hydrochlorides were prepared and purified by recrystallization to a constant melting point. Analytical results for these compounds are shown in Table 2. The average recovery of amine from these compounds is 100 per cent and the largest error in a single determination is 1 per cent.

Approximately 0.02 N solutions were prepared from commercial samples of monoethanolamine, diethanolamine, triethanolamine, tri-isopropanolamine and "amino glycol" and standardized by macro titration. After addition of excess hydrochloric acid to aliquots of these solutions

TABLE 3.—*Analysis of solutions of commercial alkanolamines*

COMPOUND	AMINE	
	ADDED*	FOUND
	mg.	mg.
Monoethanolamine	4.88	4.92
	9.76	9.76
Diethanolamine	15.9	16.0
	15.9	15.8
Triethanolamine	8.88	8.94
	17.8	17.9
	17.8	17.7
Tri-isopropanolamine	14.8	15.1
	14.8	14.9
"Amino glycol"	11.7	11.6
	11.7	11.6

\* Calculated from the results of direct macro titration.

and evaporation to dryness on the steam bath, the amine content was determined by the proposed procedure. The recoveries are shown in Table 3. The alkanolamine hydrochlorides are not volatilized or decomposed at

TABLE 4.—*Analysis of mixtures*

COMPOUNDS	QUANTITY*	TOTAL BASE†	
		FOUND	CALCULATED
Triethanolamine and sodium hydroxide	mg.		
	6.66	5.15	5.22
Monoethanolamine and ammonia	2.40	5.15	
	3.17	4.90†	4.90
	0.78	4.90† 4.92†	
Triethanolamine and borax	6.66	4.70	4.72
	9.55		
Monoethanolamine and boric acid	4.88	4.00	4.00
	10.0		
"Amino glycol" and boric acid	9.53	4.55	4.53
	10.0		

\* Quantity in aliquot taken for analysis as determined by direct macro titration.

† Hydrobromic acid used.

‡ Milliequivalents  $\times 50$ .

100°C. Hence, there is no danger of loss of amine in the determination of these substances even if the residue of amine hydrochloride is heated on the steam bath for some time after evaporation to dryness.

Metallic and ammonium soaps are also used in the preparation of emulsions and mixtures of an organic amine and an inorganic base may be en-

TABLE 5.—*Analysis of emulsions*

EMULSION NO.	INGREDIENTS	WEIGHT*	AMINE†	
			FOUND	CALCULATED
		<i>gram</i>	<i>mg.</i>	<i>mg.</i>
1	Triethanolamine	0.0666	16.7	16.7
	Stearic acid	0.5	16.8	
	Water	ca. 2.0		
2	Triethanolamine	0.0666	16.7	16.7
	Beeswax	0.25	16.6	
	Mineral oil	2.0		
	Water	ca. 2.0		
3	Triethanolamine	0.0666	13.3	13.3
	Glycerin	0.5	13.6	
	Stearic acid	0.5		
	Mineral oil	1.0		
	Water	ca. 2.0		
4	Borax	0.0955	Total Base‡	
	Triethanolamine	0.0666	4.70	4.72
	Spermaceti	0.25	4.70	
	Beeswax	0.25		
	Mineral oil	2.0		
	Water	ca. 5.00		

\* Entire sample.

† In aliquot taken for final titration.

‡ Milliequivalents  $\times 50$ .

countered. Mixtures of alkanolamines and inorganic bases were prepared and analyzed by the proposed method. Results of these experiments are shown in Table 4. As would be expected the total base was recovered in each case.

The borate salt of one of the amines has been proposed for use in cosmetics. As shown in Table 4 addition of 1–2 equivalents of boric acid to solutions of monoethanolamine and "amino glycol" did not affect the recovery of the amine. In a single experiment (not tabulated) in which a much larger excess of boric acid was added to monoethanolamine the net titration was about 0.1 ml. low.

Cosmetic emulsions ordinarily contain only small amounts, i.e. 1-2 per cent, of emulsifier. Extraction of the amine from large samples is usually rather troublesome, and for this reason it is desirable to use as small a sample as possible. If the final determination is conducted on a semi-micro scale, enough amine for duplicate or triplicate analyses may be obtained from samples of 1-4 grams. A sample of this size is sufficiently large to be representative of the entire emulsion and is ordinarily fairly easy to extract.

The efficiency of the proposed extraction procedure was tested as follows: Measured amounts of the amine and other ingredients were placed in a beaker, heated on the steam bath to 70-80°C., and stirred until thoroughly emulsified. The entire sample was then extracted and the amine determined. The results, as shown in Table 5, appear satisfactory. Other methods for separation of the amine from the emulsion, such as extraction of the oils and fatty acids from an acidified solution, may be used if desired.

The accuracy of the proposed method equals that of a nitrogen determination, but less time is required for an analysis. This saving in time is considerable when, as is often the case, it would be necessary to make a preliminary separation of the emulsion prior to the Kjeldahl digestion because of the low amine (and nitrogen) content. An obvious advantage of the proposed method is that it may be applied directly to mixtures which contain non-titrable nitrogen compounds in addition to the emulsifier.

#### SUMMARY

A semi-micro volumetric method for the determination of alkanolamines in emulsions has been presented. When an inorganic base is also present the method may be used to determine the total base.

Typical results are given.

#### DETERMINATION AND IDENTIFICATION OF 2-AMINOANTHRAQUINONE IN D&C BLUE NO. 9

By O. L. EVENSON (Cosmetic Division, Food and Drug Administration,  
Federal Security Agency, Washington, D. C.)

The intermediate 2-aminoanthraquinone is used in the preparation of D&C Blue No. 9 (3,3'-dichloroindanthrene). This color, also known as Carbanthrene Blue, is included in the list of those that may be certified by the U. S. Food and Drug Administration for use in drugs and cosmetics.<sup>1</sup>

In the proposed method 2-aminoanthraquinone is separated from the

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<sup>1</sup> "S.R.A. F.D.C. 3," Food & Drug Administration, Federal Security Agency

dye and treated with bromine, which destroys any trace of dye remaining and converts the intermediate into 2-amino-1, 3-dibromoanthraquinone. This is estimated colorimetrically. The intermediate is identified by the melting point of the bromine compound and that of its acetyl derivative.

#### METHOD

##### REAGENTS

- (a) *Ethyl alcohol*.—95%.
- (b) *Bromine water*.
- (c) *Hydrazine sulfate*.—Saturated solution.
- (d) *Standard solution of 2-aminoanthraquinone*.—Dissolve 50 mg. in 500 ml. of 95% ethyl alcohol.

##### DETERMINATION

In a tall, 300 ml. beaker dissolve 0.2 gram of D&C Blue No. 9 in about 3 ml. of  $H_2SO_4$ , using a glass rod to break up the lumps. Add *carefully* small quantities of alcohol, stirring after each addition, until 75 ml. has been added. Heat on the steam bath with occasional stirring for about 30 minutes, transfer to a 100 ml. volumetric flask, cool, make to mark with alcohol, and filter.

Pipet 10 ml. or less of the filtrate into a suitable test tube. Prepare a series of standards containing from 0.05 to 0.15 mg. of 2-aminoanthraquinone in the same volume of alcohol and acidify with 0.1 ml. of  $H_2SO_4$ . To the standards and the unknown add 0.1 ml. of bromine water, 0.1 ml. of hydrazine sulfate, and 10 ml. of water. Mix after each addition and compare the unknown with the standards.

Known quantities of 2-aminoanthraquinone in alcoholic solution were added to weighed aliquots of D&C Blue No. 9 and recoveries determined. The sample of D&C Blue No. 9, used for this purpose and taken from a certified batch, gave a negative test for the free intermediate by this method. The results in Table 1 indicate a recovery of approximately 95 per cent.

TABLE 1.—*Recoveries on samples of D&C Blue No. 9 containing known quantities of intermediate*

NO.	WEIGHT OF COLOR	2-AMINOANTHRAQUINONE		
		ADDED	FOUND	RECOVERY
	mg.	mg.	mg.	per cent
1	100	0.5	0.48	96
2	100	1.0	0.96	96
3	200	2.0	1.94	97
4	300	2.5	2.45	98
5	200	4.0	3.80	95
6	200	1.0	0.95	95
7	200	0.5	0.47	94
8	200	0.25	0.237	95
9	200	1.50	1.40	93
		Average		95.4

For the purpose of identification the melting points of the 2-amino-1, 3-dibromoanthraquinone and its acetyl derivative were obtained.

Scholl<sup>2</sup> has shown that bromination of 2-aminoanthraquinone in sulfuric acid solution with an excess of bromine leads to the formation of the dibromo derivative and that by acetylation of this product two acetyl groups are introduced.

#### IDENTIFICATION

*Preparation of 2-amino-1,3-dibromoanthraquinone.*—In a 2 liter Erlenmeyer flask dissolve 500 mg. of 2-aminoanthraquinone in 500 ml. of 95% alcohol and acidify with about 2 ml. of H<sub>2</sub>SO<sub>4</sub>. Cool to about 50°C., add 2 grams of bromine, stopper, and shake at intervals for about 15 minutes. Dilute with an equal volume of water, shake, and after about 15 minutes filter and wash with a few ml. of 50% alcohol.

The product crystallized from alcohol in orange-brown needles. When recrystallized from 95 per cent alcohol, it melted at 247°C.<sup>3</sup> (Melting Point Apparatus by K. S. Markley, stem immersed, no correction required). This was not changed by an additional recrystallization. Scholl obtained a melting point of 239, while Ullman and Medenvald<sup>4</sup> report a corrected melting point of 249.5°C. on the recrystallized product.

*Preparation of the diacetyl derivative.*—Dissolve 2-amino-1, 3-dibromoanthraquinone in acetic anhydride and reflux for about 10 hours. Dilute with water, filter, and wash with water.

The product, which separated on dilution with water, was recrystallized from 95 per cent alcohol as yellow green rods. After a second recrystallization the melting point was 211°C.<sup>3</sup> An additional recrystallization did not change this figure. This product contained 33.9 per cent of bromine.<sup>5</sup> The theoretical figure for 2-(N-diacetyl)-1, 3-dibromoanthraquinone is 34.4 per cent.

To obtain the intermediate from the color for the purpose of identification, dilute the acid alcoholic filtrate (see method) with twice its volume of water and shake with ether. Wash the solvent with a few ml. of water and evaporate to dryness. Dissolve the residue in alcohol and prepare the derivatives.

#### SUMMARY

A colorimetric method has been presented for the quantitative estimation of 2-aminoanthraquinone in D&C Blue No. 9. The intermediate is removed from the color and treated with bromine to form 2-amino-1, 3-dibromoanthraquinone, which is compared colorimetrically with standards. A 95 per cent recovery is indicated.

2-Amino-1, 3-dibromoanthraquinone and its acetyl derivative were prepared for identification of the intermediate. Their melting points were found to be respectively 247° and 211°C.

<sup>2</sup> Scholl, R., *Ber.* 40 (2), 1701 (1907).

<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 6, 475 (1934).

<sup>4</sup> Ullman, F. and Medenvald, R., *Ber.*, 46 (2), 1803, 1808 (1913).

<sup>5</sup> Clark, G. R. and Jones, J. H., *This Journal*, 26, 433 (1943).

## MODIFICATION OF PICRIC ACID METHOD FOR DETERMINATION OF HYDROCYANIC ACID IN WHITE CLOVER PLANTS\*

By J. T. SULLIVAN† (U. S. Regional Pasture Research Laboratory,  
State College, Pa.)

The method previously proposed from this Laboratory for the quantitative determination of hydrocyanic acid in white clover involved the assumption that this acid was liberated from its parent glucoside by the hydrolyzing enzyme present in the plant.<sup>1</sup> The glucoside has since been isolated by Melville and Doak<sup>2</sup> and found to be a mixture of linamarin and lotaustralin, and was shown by Coop<sup>3</sup> to be hydrolyzed by linamarase prepared either from linseed or from the clover plant. That the enzyme is not present in every plant that contains the glucoside has been brought out by the observations of Corkill.<sup>4</sup> Complete hydrolysis of the glucoside in plants deficient in the enzyme has been obtained by Melville, Coop, Doak, and Reifer<sup>5</sup> after the addition of the prepared linamarase. The present paper presents a modification of the previously published procedure and contains data which emphasize the necessity of supplementing the enzyme activity of the plant in the quantitative determination of the total potential HCN in white clover.

## MATERIAL AND METHODS

According to the work of Corkill<sup>4</sup> white clover plants may be grouped into four classes. These classes may be numbered for convenience and described as follows: I. Contains both glucoside and enzyme; II. contains the glucoside but not the enzyme; III. contains the enzyme but not the glucoside; and IV. contains neither the glucoside nor the enzyme.

Plants of all four classes were available from studies carried out in this Laboratory on the inheritance of the glucoside and enzyme.<sup>6</sup> Plants known to belong to each of these classes, as determined by qualitative or roughly quantitative tests, were increased clonally to furnish material for this quantitative study.

Linamarase solution was prepared according to the directions of Coop<sup>3</sup> and contained 0.8 per cent solids. Two samples of takadiastase, also capable of liberating HCN from its parent glucoside, were available, one an old commercial preparation and the other a fresh undiluted takadiastase.

The procedure used for the analysis was that previously described,<sup>1</sup>

\* Contribution No. 65, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the northeastern states.

† Physiologist.

<sup>1</sup> Sullivan, J. T., *This Journal*, 22, 781 (1939).

<sup>2</sup> Melville, J., and Doak, B. W., *New Zealand J. Sci. Tech.*, 22B, 67 (1940).

<sup>3</sup> Coop, I. E., *Ibid.*, 22B, 71 (1940).

<sup>4</sup> Corkill, L., *Ibid.*, 65.

<sup>5</sup> *Ibid.*, 144.

<sup>6</sup> Atwood, S. S., and Sullivan, J. T., *J. Heredity*, 34, 311 (1943).

with two exceptions: (1) the treatment previous to distillation was modified and described for each case, and (2) a glass filter with light absorption equivalent to a 10 per cent copper sulfate solution was substituted for the solution itself in the photometer. In all cases fresh leaves of white clover, including petioles, were used for an analysis, and the results are reported as milligrams of HCN in 100 grams of fresh leaves. The sample, usually 10 grams, was subjected to any treatments necessary, such as grinding and enzyme additions (described later), and placed in short-necked 500 ml. Kjeldahl flasks fitted with rubber stoppers covered with tinfoil. After a period of incubation at room temperature, the stoppers were removed; the flask was quickly fitted to the distillation apparatus; and steam distillation was carried out. An aliquot of the distillate was heated with alkaline picrate and the color change measured in a photometer.

### RESULTS

*Analysis of Various Classes of Clover.*—Some results indicating the type of data obtained from the four classes of plants described above are given

TABLE 1.—Yields of HCN (mg./100 grams) from unground clover leaves after various additions and after incubation at room temperature

CLASS	CLONE NO.	PERIOD OF INCUBATION BEFORE DISTILLING	NO ADDITIONS	ADDITION OF 5 ML. TOLUENE	ADDITION OF TOLUENE AND 1 ML. LINAMARASE	ADDITION OF TOLUENE AND 0.1 GRAM TAKADIASTASE
		<i>days</i>				
I	24(8)	1	3.0	10.7	14.3	13.8
I	24(23)	1	—	8.8	12.6	—
II	25(20)	1	—	0.9	7.0	—
II	25(10)	7	—	2.0	18.8	—
III	25(19)	None	—	0.8	—	—
III	25(19)	1	—	—	1.2	—
IV	24(2)	None	—	0.6	—	—
IV	24(2)	1	—	—	1.1	0.9

in Table 1. These results were obtained with unground leaves to which the additions given in the table were made. In Class I, although natural enzyme was present, the yield was increased during short periods of incubation by added linamarase or takadiastase. Very low yields were obtained in Class II unless enzyme was added. Classes III and IV, lacking in HCN in qualitative tests which determined the class, yielded small amounts with steam distillation both with and without the addition of enzyme. One preparation of linamarase subjected to distillation gave a yield of approximately 0.001 mg. of HCN per ml., an insufficient amount to account for the yields obtained. The other preparation of linamarase and the takadiastase preparations yielded no HCN. It may be that Classes III and IV



are not entirely free from HCN, or that some other substance present in minor amounts gives the same reaction. Doak<sup>7</sup> suspected the presence of a volatile substance, which may have been an unsaturated acid, interfering in the determination of HCN in clover by an iodine or silver nitrate titration of the steam distillate. The presence of hexenal in the steam distillate from cut leaves also suggests a possible interference. In the absence of definite proof of an interfering substance in the picric acid procedure it may be concluded that these representatives of Classes III and IV which were studied contain minute amounts of HCN not detected by the qualitative method.

Since Classes III and IV are of no great interest in this phase of the problem, only Classes I and II will be considered further.

*Conditions for Maximum Yield in Classes I and II.*—In Class II, it is obvious that added enzyme is essential for the recovery of HCN from the plant. In Class I added enzyme may hasten the complete hydrolysis of the glucoside. Studies were made, therefore, within these two classes of the effect of various treatments of the leaves before incubation, such as addition of enzyme, addition of toluene, freezing and thawing of the leaves, grinding in a mortar with sand and disintegration in a Waring blender, and also on the duration of the incubation. Any additions, when made, followed any grinding and were in the following amounts; toluene 5 ml.; linamarase 1 ml., occasionally 5 ml.; takadiastase 0.1 gram of solid. Freezing was accomplished by placing the stoppered flask inside the freezing unit of a refrigerator until the contents were frozen solid. When enzyme addition was combined with freezing, the enzyme was sometimes added before, and sometimes after, freezing. The grinding with sand in a mortar required several minutes, after which the leaf remnants were transferred to the flask with the aid of distilled water from a wash bottle. Disintegration of the leaves under water in the blender seemed to be as complete as necessary in less than one minute, and in this case also the remnants were transferred to the flask with water. If no grinding was resorted to, about 25 ml. of water was always added to aid the distribution of added enzyme.

The results of some comparisons are given in Table 2. Because of insufficient plant material and equipment all determinations were not made at the same time and valid comparisons may thus be made only within samples harvested at the same time. For convenience, the determinations being compared are grouped as an "experiment."

With Class I, which contained plant enzyme, no HCN was evolved from untreated leaves, but the addition of toluene alone caused the evolution of the greater part but not all of the HCN; a still greater yield was obtained with added enzyme. In Experiment 2 only 19.5 mg. of HCN was obtained after 11 days' incubation with toluene as compared with 23.5

<sup>7</sup> *New Zealand J. Sci. Tech.*, 20A, 163 (1938).

TABLE 2.—Yields of HCN (mg./100 grams) from clover leaves after various treatments and after incubation at room temperature

CLASS	EXPT.	CLONE	TIME INCUBATION	TREATMENT PREVIOUS TO INCUBATION—						
				NO TREATMENT	TOLUENE ONLY	GRINDING ONLY	GRINDING AND TOLUENE	TOLUENE AND ENZYME	FREEZING, TOLUENE, AND ENZYME	GRINDING, TOLUENE, AND ENZYME
I	1	12(30)	1 day		14.3			18.6 <sup>1</sup>	17.0 <sup>2</sup>	
	2	12(30)	2 days 11 days		19.5			23.5 <sup>1</sup> 25.0 <sup>1</sup>	25.0 <sup>1</sup> 23.0 <sup>1</sup>	
	3	12(30)	None 5 hours 1 day 11 days	1.0		6.0 <sup>3</sup>	32.4 <sup>4</sup> 40.0 <sup>4</sup>			7.6 <sup>1,5</sup> 31.0 <sup>1,5</sup> 38.0 <sup>1,5</sup> 38.0 <sup>1,5</sup>
	4	25(13)	None 1 day 3 days		1.3 6.2 7.0					
	5	24(8)	3 days 3 days	None	9.6			16.0 <sup>1</sup> 10.6 <sup>2</sup>	16.2 <sup>1</sup>	
	6	24(8)	4 hours 1 day 3 days				17.5 <sup>4</sup> 21.2 <sup>4</sup> 22.0 <sup>4</sup>			19.0 <sup>1,4</sup> 21.2 <sup>1,4</sup> 22.0 <sup>1,4</sup>
II	7	26(13)	1 day 4 days 8 days 19 days		3.1			7.9 <sup>1</sup> 10.8 <sup>1</sup> 12.2 <sup>1</sup> 12.8 <sup>1</sup>		
	8	26(13)	1 day 3 days 3 days 15 days					11.8 <sup>1</sup>		11.5 <sup>1,4</sup> 4.5 <sup>2,4</sup> 11.7 <sup>1,4</sup> 12.0 <sup>1,5</sup>
	9	25(10)	5 hours 1 day 5 days					22.0 <sup>1</sup>		16.0 <sup>1,4</sup> 21.8 <sup>1,4</sup> 22.0 <sup>1,4</sup>
	10	25(10)	None 4 hours 1 day 11 days							1.5 <sup>1,5</sup> 11.0 <sup>1,5</sup> 20.5 <sup>1,5</sup> 22.7 <sup>1,5</sup>

<sup>1</sup> Linamarase.<sup>2</sup> Undiluted takadiastase.<sup>3</sup> Commercial takadiastase.<sup>4</sup> Grinding in mortar with sand.<sup>5</sup> Grinding in blender.

mg. after only two days' treatment with toluene and linamarase. A similar comparison may be made in Experiment 5. Little advantage was observed in freezing the leaves. Grinding, when combined with incubation with toluene, caused a rapid attainment of the maximum HCN yield. Additional yield was not obtained by adding enzyme to ground leaves when the incubation was maintained for at least one day.

In Class II, enzyme was essential and the necessity of grinding only has been emphasized. Without grinding (Experiment 7) the yield continued steadily beyond the eighth day, but with grinding (Experiments 8, 9, and 10) the maximum was reached, or nearly so, in one day.

The source of enzyme appeared to make some difference in the results. Takadiastase, though added in much larger amounts than linamarase, sometimes gave a lower yield of HCN than did linamarase. The method of grinding, on the other hand, appeared to make no difference. Since grinding in the blender was quicker and less laborious, it was favored.

To determine whether a loss in HCN occurred while the plant was being disintegrated in the blender, four 10-gram samples of clone 24,<sup>5</sup> Class I, were gathered. Two samples were immediately placed in flasks with added enzyme and toluene, and the flasks were stoppered. The remaining two samples were ground in the blender, washed into flasks, and after enzyme and toluene had been added the flasks were stoppered. All four samples were stored before distillation for 10 days, a period of time believed sufficient to cause the maximum hydrolysis of the glucoside, even with unground leaves. On distillation, the unground samples yielded 22.7 and 21.9 mg. of HCN per 100 grams and the ground samples 22.2 and 22.7 mg. Thus no loss of HCN may be assumed to have occurred in the grinding process.

The time of incubation necessary for maximum yields, after grinding and addition of enzyme, varied slightly in different experiments. In Experiment 3 one day was sufficient; in Experiments 6, 8, 9, and 10 only slight increases were observed beyond one day. Incubation at 37°, as used by Melville, *et al.*,<sup>5</sup> may hasten the hydrolysis. However, no studies were made on the effect of the temperature of incubation or on the influence of the hydrogen-ion concentration.

From the above data it appears that the addition of an enzyme preparation should be incorporated into the procedure for determining HCN in white clover, and that disintegration of the leaves is essential if the maximum yield of HCN is to be obtained after a reasonably short digestion period. Since the class to which a particular plant belongs cannot be determined without preliminary testing, enzyme should be added in all determinations if the total quantity of HCN is the object of the analysis.

#### MODIFIED PROCEDURE

Grind 10 grams of fresh clover leaves with water in a Waring blender or with sand, in a mortar. Transfer the liquid and the leaf fragments to a flask, add 1 ml. of linamarase solution and a few ml. of toluene, stopper the flask tightly, and allow the sample to incubate for at least 1 day. Remove the stopper, immediately connect the flask to a distilling apparatus, and steam distil 80–90 ml., catching the distillate in a beaker containing 5 ml. of 2% KOH. During the first half of the distillation have the tip of the condenser dipping below the surface of the liquid in the beaker. Heat

<sup>5</sup> Nye, W., and Spoehr, H. A., *Arch. Biochem.*, 2, 23 (1942).

an aliquot of the distillate with alkaline picrate solution and compare the color change with a blank determination by means of a photometer as previously described.<sup>1</sup>

*Addition of Enzyme to Sudan Grass.*—Hydrocyanic acid in Sudan grass has been determined by the same procedure as used with white clover. To determine the influence of added enzyme, a composite sample of Sudan grass leaves was taken from a number of plants, cut into small pieces with shears, and divided into 10-gram lots, and to some lots additions were made as before. They were stored without further grinding. When toluene only had been added, the yield of HCN per 100 grams of leaves increased from 21.5 at two days' incubation to 26.0 mg. at 13 days. When linamarase as well as toluene had been added, the yield increased from 25.0 mg. at two days to 26.4 mg. at 13 days. With added takadiastase a sample stored 13 days yielded 27.5 mg. of HCN. Hence the addition of enzyme to unground leaves caused slightly greater yields of HCN for the same period of incubation time.

#### SUMMARY

A modification of a previously published procedure for the determination of HCN in white clover is described. Disintegration of the leaves by grinding, addition of a quantity of linamarase as well as toluene, and incubation in a closed flask for at least one day before distillation are advised.

### IMPROVED DITHIZONE PROCEDURE FOR DETERMINATION OF ZINC IN FOODS

By O. R. ALEXANDER and L. V. TAYLOR\* (American Can Company, Research Department, Maywood, Ill.)

The present tentative microcolorimetric procedure<sup>1</sup> for the determination of zinc in foods has been subject to criticism for numerous reasons. Collaborative studies have shown appreciable variations in results obtained by the method, which may have been due partially to relative inexperience of the collaborators in working with dithizone procedures or to inherent shortcomings of the method. With the more or less common criticisms in mind the authors undertook the problem of devising a more suitable dithizone procedure for zinc in foods to be ultimately subjected to collaborative work prior to its recommendation to the Association of Official Agricultural Chemists for acceptance as official.

The revised procedure involves the wet oxidation of the sample; elimination of lead, copper, cadmium, bismuth, antimony, tin, mercury, and silver as sulfides with added copper as a scavenger agent; a simultaneous

\* Associate Referee for Zinc Methods.

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1940, 415.

elimination of cobalt and nickel by extracting the metal complexes of a -nitroso-b-naphthol and dimethyl-glyoxime, respectively, with chloroform; extraction of the zinc dithizonate with carbon tetrachloride; transfer of the zinc to dilute hydrochloric acid; and a final extraction of the zinc dithizonate for color measurement.

Holland and Ritchie,<sup>2</sup> and Cowling<sup>3</sup> have reported some difficulty in dissolving the zinc in the ash of some products. There have also been instances in previous work in which loss of zinc during dry ashing was suspected. This question has been eliminated in the revised technic. It has been the experience at this laboratory that the contamination due to the use of the relatively large volumes of oxidizing acids need not be serious if the reagents are selected with reasonable care from reliable sources. It is considered that the ever-present danger of loss due to volatilizing during dry ashing or to fixation of the zinc on the containing dish or in the ash itself is more difficult to overcome than any contamination due to acids used in the wet oxidation.

The workers mentioned previously have used the diethyldithiocarbamate competitive complex in combination with mixed color dithizone technics to remove the interference of certain dithizonate-forming metals. Cowling and Miller<sup>4</sup> have shown that when zinc is extracted with dithizone from an aqueous solution containing carbamate, part of the zinc exists as the carbamate and resists extraction as the dithizonate. By keeping constant all the factors pertaining to the distribution of zinc between the carbamate and dithizone complexes as well as the distribution of excess dithizone between the aqueous and solvent phases, the zinc concentration-light transmission relationship is reproducible. The introduction of this rigid empiricism, however, is not entirely favored. Use of carbamate and its resultant objections is obviated in the proposed revision by employing a sulfide separation to eliminate some of the dithizonate-forming metals.

In the procedures devised by Cowling,<sup>3</sup> Holland and Ritchie,<sup>2</sup> and earlier investigators, P. L. Hibbard,<sup>5</sup> and E. B. Sandell,<sup>6</sup> the elimination of cobalt, nickel, cadmium, and bismuth is not carried out in an entirely satisfactory and convenient manner. The proposed technic has yielded accurate and reproducible results in the presence of all interfering elements commonly found in food products.

The questionable zinc contamination due to extraction from Pyrex glassware has been a persistent source of difficulty in all dithizone zinc methods employing alkaline extractions. No progress was made by the authors toward eliminating this source of error. The problem was taken

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<sup>2</sup> *This Journal*, 24, 348 (1941).

<sup>3</sup> *Ibid.*, 520.

<sup>4</sup> *Ind. Eng. Chem., Anal. Ed.*, 13, 145 (1941).

<sup>5</sup> *Ibid.*, 9, 127 (1937).

<sup>6</sup> *Ibid.*, 484.

up with the glassware manufacturers, but to date no apparent steps have been taken toward its solution. From past experience, however, this difficulty may be reduced so that it is of little consequence provided the proper precautions are observed.

The details of the method follow:

#### ZINC IN FOOD PRODUCTS

##### *Spectrophotometric Method*

(All water employed throughout must be redistilled from glass.)

#### REAGENTS

(a) *Nitric acid*.—C.P., concentrated (should be redistilled if appreciably contaminated, although not usually necessary).

(b) *Sulfuric acid*.—C.P., concentrated (should be tested if zinc contamination is suspected).

(c) *Ammonium hydroxide*.—C.P., concentrated (should be redistilled if appreciably contaminated).

(d) *Copper sulfate solution*.—Dissolve 8 grams of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in water and dilute to 1 liter. 1 ml. contains 2 mg. of copper.

(e) *Ammonium citrate solution*.—Dissolve 225 grams in water and make alkaline to phenol red with  $\text{NH}_4\text{OH}$  and add 75 ml. excess. Dilute to 2 liters. This solution should be extracted immediately before use as follows: Add an excess of dithizone and extract with  $\text{CCl}_4$  until the solvent layer is a clear bright green. Remove excess dithizone by repeated extraction with  $\text{CHCl}_3$ , and finally extract once more with  $\text{CCl}_4$ . It is essential that excess dithizone be entirely removed, otherwise zinc will be lost during the elimination of cobalt and nickel.

(f) *Dimethylglyoxime solution*.—Dissolve 2 grams of the reagent in 10 ml. of  $\text{NH}_4\text{OH}$  and 200–300 ml. of water, filter, and dilute to 1 liter.

(g) *a-Nitroso-b-naphthol solution*.—Dissolve 0.25 gram in  $\text{CHCl}_3$  and dilute to 500 ml.

(h) *Chloroform*.—Redistilled.

(i) *Diphenylthiocarbazone (dithizone)*.—Dissolve 0.125 gram in 5 ml. of  $\text{NH}_4\text{OH}$ , add 250 ml. of water, and extract repeatedly with  $\text{CCl}_4$  until the solvent layer is a clear, bright green color. Discard the solvent layer and filter the aqueous portion through a washed ashless paper. This solution is best prepared as needed since it is only moderately stable, even when kept in the dark and under refrigeration.

(j) *Carbon tetrachloride*.—Redistilled.

(k) *Hydrochloric acid*.—0.04 N. Dilute the required amount of concentrated C.P. acid with water (redistilled acid may be used although not usually required).

(l) *Standard zinc solution*.—Dissolve 0.500 gram of pure granulated zinc in a slight excess of dilute  $\text{HCl}$  and dilute to 1000 ml. For use dilute 10 ml. of this stock solution to 1000 ml. with 0.04 N  $\text{HCl}$ . 1 ml. of this working standard contains 5 micrograms of zinc.

#### DETERMINATION

*Preparation of Sample*.—Weigh a representative sample not exceeding 25 grams of material, estimated to contain 25–100 micrograms of zinc, into a 250 ml. Erlenmeyer flask. If the sample is a liquid, evaporate to a small volume. Add concentrated  $\text{HNO}_3$  and heat cautiously until the first vigorous reaction subsides somewhat, then add 5 ml. of  $\text{H}_2\text{SO}_4$ . Continue heating, adding more  $\text{HNO}_3$  in small increments if necessary to prevent charring, until fumes of  $\text{SO}_3$  are evolved and the solution remains clear and almost water white. Add 0.5 ml. of  $\text{HClO}_4$  and continue heating

until the  $\text{HClO}_4$  has been almost completely removed. Allow to cool and dilute to about 40 ml.

*Separation of Sulfide Group.*—To the  $\text{H}_2\text{SO}_4$  solution add 2 drops of methyl red indicator and 1 ml. of the  $\text{CuSO}_4$  solution, and neutralize with concentrated  $\text{NH}_4\text{OH}$ . Add sufficient  $\text{HCl}$  to make the solution approximately 0.15 *N* with respect to this acid (approximately 0.5 ml. excess in 50 ml. of solution is satisfactory). The *pH* of the solution at this point as measured with a glass electrode is 1.9–2.1. Pass in  $\text{H}_2\text{S}$  at a moderately rapid rate until precipitation is complete. Filter through a fine-textured paper (Whatman No. 42 or equivalent) into a 250 ml. beaker and wash with 3 or 4 small portions of water. Boil the filtrate until the  $\text{H}_2\text{S}$  has been completely expelled. A few ml. of bromine water will serve to oxidize the last traces of  $\text{H}_2\text{S}$ . Dilute the resulting solution to 100 ml.

*Elimination of Nickel and Cobalt.*—Transfer a 20 ml. aliquot of the prepared solution to a 125 ml. separatory funnel, add 5 ml. of the ammonium citrate buffer, 2 ml. of the dimethylglyoxime solution, and 10 ml. of the  $\alpha$ -nitroso- $\beta$ -naphthol solution and shake for 2 minutes. Discard the solvent layer and extract with 10 ml. of  $\text{CHCl}_3$  to remove the residual  $\alpha$ -nitroso- $\beta$ -naphthol. Discard the solvent layer.

*Isolation and Estimation of Zinc.*—To the aqueous phase following the removal of nickel and cobalt, which at this point has a *pH* of 8.0–8.2, add 2.0 ml. of the dithizone solution and 10 ml. of  $\text{CCl}_4$ , and shake for 2 minutes. Allow the phases to separate, and remove the aqueous layer as completely as possible, withdrawing the liquid by means of a pipet attached to a vacuum line. Add approximately 25 ml. of water, and without shaking again draw off the aqueous layer. Add 25 ml. of the 0.04 *N*  $\text{HCl}$  and shake for 1 minute to transfer the zinc to the acid-aqueous layer. Draw off and discard the solvent, being careful to dislodge and remove the drop that usually floats on the surface. To the acid solution add 5.0 ml. of the ammonium citrate solution and 10.0 ml. of  $\text{CCl}_4$ . The *pH* of the solution at this point is 8.8–9.0. Determine the amount of dithizone to be added as follows: To a separatory funnel containing 4.0 ml. of the working zinc standard (20 micrograms) made up to 25 ml. with the 0.04 *N*  $\text{HCl}$ , 5.0 ml. of the citrate buffer, and 10.0 ml. of  $\text{CCl}_4$ , add the dithizone reagent in 0.1 ml. increments, shaking briefly after each addition until a faint yellow color in the aqueous phase indicates a bare excess of the reagent. Multiply the volume of dithizone solution required by 1.5 and add this volume (to the nearest 0.05 ml.) to all samples. Shake for 2 minutes. By means of a pipet transfer exactly 5.0 ml. of the solvent layer to the spectrophotometer cell, dilute with 10.0 ml. of  $\text{CCl}_4$ , and determine the spectral transmission at 530  $\text{m}\mu$ . (The dilution may be made in a clean, dry test tube if the design of the cell does not permit mixing directly. A Coleman Universal Model 11 spectrophotometer with square test tube cuvettes was used throughout this investigation.)

*Preparation of Standard Curves.*—Prepare a series of separatory funnels containing 0, 5, 10, 15, and 20 micrograms of zinc made up to 25 ml. with the 0.04 *N*  $\text{HCl}$ , add 5.0 ml. of the citrate buffer, and proceed with the final extraction of zinc as directed in the previous paragraph. Plot the transmittance on a logarithmic scale against concentration and draw a smooth curve through the points. (The intercept of this curve may vary slightly from day to day, depending on the actual concentration of dithizone employed in the final extraction, but the slope should remain essentially the same.)

## DISCUSSION

*Zinc Contamination.*—It is impossible to overemphasize the importance of proper cleaning of glassware and the precautions that must be observed in guarding against contamination. All glassware should be cleaned by

immersion in boiling nitric acid followed by thorough rinsing. Analysis of samples of Pyrex glassware revealed the presence of approximately 10 p.p.m. of Zn. Whether the samples analyzed were typical of all Pyrex glass is of course unknown.

It has been demonstrated in this laboratory that it is possible to carry blank determinations through the three steps involved in the isolation and determination of zinc with very little contamination. A set of 24 blanks carried through these three separations gave an average of 0.23 microgram of zinc. All values fell within a range of 0.14 to 0.42, with an average deviation from the mean of  $\pm .06$  microgram. Estimation of these traces of zinc was carried out in the following manner: A portion of the 10 ml. of the final carbon tetrachloride extract containing the zinc was drawn off and the transmission was measured at 530 and at 620  $m\mu$ . The funnel was again shaken after addition of 0.25 ml. of 40 per cent W/V potassium cyanide solution. The remainder of the solvent layer was pipetted off and read at 530 and at 620  $m\mu$  as before. Comparison of the transmission values at 620  $m\mu$  before and after the potassium cyanide separation showed that the addition of potassium cyanide was without effect on the amount of dithizone extracted. Any increase in the transmission at 530  $m\mu$  could then be attributed to removal of zinc or to some other dithizone-forming metal. This procedure may not be entirely specific for zinc, nevertheless it does give valuable information in revealing the upper limit to the amount of zinc which may be present. This procedure was also found to be very useful in determining the efficiency and completeness of the two dithizone extractions for isolating and determining the zinc.

Another source of contamination that has been encountered is associated with the use of rubber bands or rubber "tie ons" to secure the stopper to the funnel. Such contamination may be very serious and for this reason the authors have dispensed with the use of these ties entirely and prefer to remove the stoppers, placing them in order on a glass plate.

*Interferences.*—This procedure satisfactorily eliminates all interfering elements in concentrations at which they could reasonably be expected to occur in food products. The quantities of dimethylglyoxime and of *a*-nitroso-*b*-naphthol have been shown to completely remove 100 micrograms each of nickel and cobalt in the aliquot taken for analysis with no loss of zinc. The sulfide separation has been shown to completely remove lead and all other dithizone-forming elements in the acid sulfide group so that it is almost certain that there is no compensation of errors leading to misplaced confidence in the results reported.

The amount of zinc remaining in the aqueous phase following each of the two dithizone separations was determined by the potassium cyanide separation previously described. It has been shown that these separations at the pH level which obtains (namely 8.0–8.2 in the preliminary isolation, and 8.8–9.0 in the final extraction) and at the specified concentration of



dithizone are at least 98 per cent complete. It is considered that drawing off the aqueous phase following the preliminary separation of zinc is less likely to result in loss of zinc than is to be expected in the usual procedure of transferring the solvent phase through the bore of the stopcock. In addition there is an added advantage in decreasing the number of neces-

TABLE 1.—*Recovery of zinc from sulfuric acid solutions*

ZINC ADDED micrograms	OTHER METALS ADDED	ZINC FOUND micrograms	PER CENT OF THEORETICAL
10	Cu—2 mg.	10.0	100
20		19.6	98
40		40.0	100
5	Cu—2 mg.; Ag, Bi, Cd, Hg, Ni, Pb, Sb, Sn, 0.2 mg. each	4.8	96
10		9.8	98
20		19.3	96.5
40		40.4	101

TABLE 2.—*Recovery of zinc added to fruit cocktail*

ZINC ADDED*	ZINC FOUND	THEORETICAL ZINC PRESENT	PER CENT OF THEORETICAL
p.p.m.	p.p.m.	p.p.m.	
0	2.20	2.18	—
0	2.15	2.18	—
0	2.20	2.18	—
0	2.15	2.18	—
1.0	3.34	3.18	105
2.0	4.05	4.18	97
3.0	5.15	5.18	99
4.0	6.38	6.18	103
6.0	8.34	8.18	102
7.28	9.73	9.46	103
10.0	12.4	12.2	102
10.2	12.4	12.4	100
20.0	22.3	22.2	101

\* Other metals added in each case (p.p.m.): Cu, 200; Ag, Bi, Cd, Co, Hg, Ni, Pb, Sb, and Sn, 20 each.

sary transfers with the accompanying chance of loss and/or contamination, and in effecting a decrease in the number of funnels required for a series of determinations.

*Recovery Determinations.*—Recovery determinations of two types have been made; first the determination of zinc added to a sulfuric acid solution containing copper and 0.2 milligram of each of the interfering elements listed; and second the determination of zinc added to a food product, in this case fruit cocktail, to which was added copper and 0.2 milligram of each of the interfering elements. Table 1 shows the recovery of zinc added to sulfuric acid solutions containing copper and other metals. The values

shown were corrected for the zinc present in the reagent blank, which amounted to 1.2 micrograms.

Table 2 shows the recovery of zinc which was added to 10 grams of puréed fruit cocktail, and to which had also been added copper to the extent of 200 p.p.m. and silver, bismuth, cadmium, cobalt, mercury, nickel, lead, antimony, and tin, each to the extent of 20 p.p.m. These recovery values are in the main quite satisfactory and indicate that zinc can be successfully determined by the method which has been presented, even in the presence of these relatively high concentrations of interfering elements. The authors plan to subject this method to collaborative study during the coming year.

Acknowledgment is made of the helpful suggestions given by H. J. Wichmann, Referee on Metals in Foods, and for the assistance of N. J. Linde and E. D. Sallee, who shared in the investigational work.

## METHOD FOR IDENTIFICATION OF COW MANURE IN DAIRY PRODUCTS

By REO E. DUGGAN (Food and Drug Administration, Federal  
Security Agency, New Orleans, La.)

The main filth elements which have been found in milk are manure, flies, ants and other insects, feed particles, and nondescript dirt. Under the microscope the various insects and their parts are easily recognizable, but manure particles of plant origin cannot be easily differentiated from feed fragments, which usually are fragments of hay, grasses, or grains. Therefore, this study was undertaken to determine if differences exist between digested and undigested plant materials. A positive method for differentiating the digested and undigested fragments is desirable because fecal material in food products is infinitely more repulsive to the consumer than are particles of stock feed.

Two samples of cow manure were first studied, one air-dried and the other in its original moist state. It was observed that these manures consisted of plant particles (which had survived digestion) bound together with a dark brown gummy mass. By mixing these samples with milk it was determined that the visible filth filtered out of milk on sediment pads or filter cloths represented approximately 12 per cent of the original wet manure incorporated in the milk; the rest of the manure remained in solution.

Since the microscopic size and the paucity of the foreign particles found in most foods preclude the determination of the chemical constituents—*e.g.* starch, crude fiber, and nitrogen—the use of a dye which would show the presence or absence of cell nutrients in the plant particles was inves-

tigated. A number of stains have been recommended for use in plant histology and cytology.<sup>1</sup> After testing a number of stains, Fast Green FCF (FD&C Green No. 3) was selected as showing the most promise for differentiating between cow manure particles and feed fragments. Plant material from which the cell nutrients had not been removed by digestion took the stain well, while manure particles were left unchanged from their original brown color.

The following method for differentiation calls for considerable experience on the part of the operator gained through the examination of authentic manures and feeds. Manure fragments longer than 2 mm., although recognizable by other characteristics, stain to a degree which might confuse any but the most skilled operators. A systematic investigation was made to determine the optimum conditions for each operation in the procedure.

#### PROCEDURE

Filter 500 ml. of fluid milk through a conventional sediment pad. For the isolation of insoluble filth elements from other dairy products, such as cheese, evaporated and condensed milks, etc., select a method<sup>2</sup> using sodium oxalate, sodium citrate, or diluted nitric or phosphoric acid. Using tweezers, and exercising care to prevent the inclusion of hairs, insects, nondescript dirt, etc., transfer approximately 100 vegetable fragments (max. length 2 mm.), selected at random, to a small (30 ml.) casserole\* containing 3-4 ml. of water. Transfer fragment sample to a Gooch crucible fitted with a circle of hard-surfaced filter paper (Sharkskin), using a stream of hot water. Wash thoroughly with a stream of hot (approximately 90°C.) water, then with hot 95% alcohol, and finally rewash with hot water. With tweezers transfer the fragments back to the casserole, containing a drop of water. Add, dropwise, 10 drops of 1% aqueous Fast Green FCF. Push any floating fragments beneath the surface of the staining solution to insure wetting. Stain 15 minutes (within  $\frac{1}{2}$  minute), and promptly wash the particles free of the staining solution by filtering onto a Gooch crucible, using a fine stream of cool water. Transfer the particles back to the casserole, using a *very fine* stream of water to wash particles from filter paper. Restrict water to 3-5 ml. Bring to boil (do not permit particles to adhere to sides of casserole as charring may result) and allow to boil 15 seconds (not longer). Filter immediately through a Gooch crucible, using a stream of water to rinse all fragments into the crucible. Using tweezers, transfer the fragments to casserole, containing a drop of water. Repeat staining and boiling procedure and filter immediately in a Büchner funnel through a 7 cm. filter paper (cross-sectioned in 6 mm. squares). Allow paper to dry and place in a Petri dish containing a few ml. of mineral oil. Segregate the manure fragments from all others, using low-power microscope (approximately 10 $\times$ ). Count, and report number and percentage of manure particles on the following basis of identification: Manure fragments of plant origin after staining have a characteristic dirty "worn" appearance and a brownish translucent color. They may or may not be slightly tinged with green. The presence of irregular, patternless, amorphous specks on the surface of the plant fragments is additional positive evidence of a manure origin. The majority of plant fragments from manure

<sup>1</sup> Conn, H. J., "Biological Stains," Biotech Publications, Geneva, N. Y. (1940).

<sup>2</sup> Microanalytical Methods, Food & Drug Adm. This compilation is not in general distribution but the procedures are available at any laboratory of the Food & Drug Adm. Many State food laboratories and the dairy industry have similar procedures available.

\* G. E. Keppel, U. S. Food & Drug Administration, Minneapolis, suggested the substitution of a fritted-glass filter in place of the Gooch crucible and casserole in all operations except boiling.

have these surface specks, but their absence does not preclude the particles being manure. Undigested plant fragments have a typical vivid green color, and/or other outstanding characteristics, *e.g.*, the surfaces are relatively smooth and clean, and the edges are usually sharp and not frayed or worn.

### STAINING OF AUTHENTIC FEEDS AND MANURES

Since a small number of fragments from authentic feeds did not take the stain and a few manure fragments were stained, a large number of samples were examined to determine the applicability of the method to feeds and manures from various sections of the country.

Eighty-four samples of feedstuffs, representing all types of feed available from farm and commercial sources and 14 samples of cow manure were examined. In order to conduct the experiment as objectively as possible, the fragments were judged only as "stained" or "unstained." Unstained material in authentic feeds was found to average 3 per cent and to vary from 0 to 15 per cent. The unstained fragments, in general, were from stalks of corn, sorghum, and similar material, and because of their very different appearance, as described in the procedure, could not be confused with manure fragments. The stained material in manure samples was found to average 17 per cent and to vary from 5 to 30 per cent. However, for reasons already given, it is unlikely that stained manure fragments could be confused with undigested fragments.

### COLLABORATIVE STUDY

The method was submitted to collaborative study for the purpose of obtaining information on the reliability of the method in the hands of other chemists, and of detecting possible flaws in the instructions not readily apparent to one fully familiar with the technic.

Two groups of collaborators tried the method. Those whose results are given in Table 1 had studied the method with the writer. With the excep-

TABLE 1.—*Collaborative results of analysts that had studied method*

		ANALYST V		ANALYST W		ANALYST X		ANALYST Y		ANALYST Z		MEAN	
SAM- PLE	MA- NURE ADDED	NO. FRAGS. EXAM- INED	MA- NURE FRAGS.	NO. FRAGS. EXAM- INED	MA- NURE FRAGS.	NO. FRAGS. EXAM- INED	MA- NURE FRAGS.	NO. FRAGS. EXAM- INED	MA- NURE FRAGS.	NO. FRAGS. EXAM- INED	MA- NURE FRAGS.	DEV. FROM MEAN	AV. ERROR
	<i>per cent</i>		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>		
A	75	101	65	100	72	94	71	94	69	96	59	± 4	-9
B	25	100	17	98	24	97	25	104	21	86	22	± 2	-4
C	40	101	41	100	40	102	56	105	36	107	37	± 2	± 2
D	50	109	49	106	49	89	57	96	46	79	49	± 1	-2
E	50	179	56	139	49	118	65	106	43	108	51	± 4	± 4
F	0	135	4	137	5	136	18	109	5	143	7	+1	—
Average Error			± 5		-1				-5		± 5	± 2	± 4

tion of Analysts 1 and 2, Table 2 reports results by chemists who had had no previous training in the application of the method. They obtained these results only after thorough study of authenticals.

TABLE 2.—*Manure found by untrained analysts*

COLLABORATOR	SAMPLE 1	SAMPLE 2	SAMPLE 3
		Manure Added (per cent)	
	0	100	51
		Manure Found (per cent)	
1	0.5	97.0	43
2	0	98	49
3	1	97	49.5
4	0	97	50
5	1.5	98.3	49.8
6	2	100	53
7	2	95	54
Average	1.0	97.5	49.8

Thirty determinations, representing 6 samples whose composition was unknown to collaborators, are reported in Table 1. As Analyst X had had only limited background training in examining authentic manures, his results were not used in the conclusions drawn from Table 1. Examination of his segregated manure particles showed a lack of consistency in classifying the fragments and obviously his results were in error, since the other collaborators were in close agreement. His results are given to illustrate the need of experience in the identification technic.

Three of the collaborators noted that each individual fragment should be touched with a teasing-needle for the most accurate count. The use of a hand tally or counting machine gave erratic counts.

Sample F contained no manure, and the staining value for the four analysts was consistent within 1 per cent. The distribution of the remaining 20 determinations on manure bearing samples was as follows:

Number of values greater than percentages of added manure	3
Number of values equal to percentage of added manure	1
Number of values lower than percentage of added manure	16

Two of the three high determinations were reported by one collaborator on Sample C, containing 40 per cent manure, and Sample E, containing 50 per cent manure, and the percentages reported were 41 and 56 per cent, respectively. The other high result was obtained on Sample E, with 51 per cent manure fragments reported. It will be noted that the results are in excellent agreement, with an average error of  $\pm 4$  per cent.

The second series of collaborative sediment pads (Table 2) was prepared and submitted to 5 laboratories located in as many different cities.

These pads were prepared by placing the feed, manure, or mixture of feed and manure in milk and allowing to stand overnight. The samples were withdrawn with a Langsenkamp Sediment Tester and preserved with a few drops of saturated alcoholic mercuric chloride. Sub. 1 was prepared from pure alfalfa silage; Sub. 2 was prepared from straight manure; and Sub. 3 was made from a mixture of 49 per cent alfalfa silage and 51 per cent manure, by count. The results are excellent.

#### EFFECT OF COMMERCIAL FOOD MANUFACTURING PROCESSES ON FEEDS AND MANURES

The effect of the various enzymatic and bacterial actions involved in the manufacture of cheese was determined by the addition of alfalfa hay to milk, which was then made into cheese by the usual cheddar process. The cheese was aged for 10 weeks. Three common methods<sup>2</sup> using chemical agents (sodium citrate, sodium oxalate, and phosphoric acid) were used for the isolation of the hay from the cheese. Evaporated and condensed milks were prepared in the laboratory from contaminated fresh milk. The above treatments had no effect upon the differential staining of feeds and manures.

#### DISCUSSION

By use of data obtained in the examination of authentic feeds and manures it is possible to compare the results obtained by arbitrarily counting only stained and unstained fragments with those obtained by experienced operators using every means of differentiation at their command. It is possible to use the staining as a sole criterion, but it is far preferable, and more nearly accurate, to rely upon an experienced analyst's judgment. As has been previously pointed out, the unstained fragments found in authentic feed are of a type not easily confused with manure.

#### SUMMARY AND CONCLUSIONS

The technic of staining undigested vegetable fragments with Fast Green FCF has been adapted to the routine determination of digested plant particles (manure) in milk and related products.

The procedure has been used in the examination of 84 feed samples and 14 manure samples involving 441 determinations and 83,899 vegetable fragments. It has also been successfully used in the collaborative study of laboratory-prepared unknowns by 8 chemists.

The following conclusions have been reached:

- (1) For best results it is necessary to acquire a thorough knowledge of the appearance of various authentic feeds and manures after being subjected to the staining procedure, and to apply all of this knowledge in segregating the manure fragments from undigested plant material.

- (2) The staining characteristics of plant material are not affected by cheese or evaporated milk manufacturing practices nor by the usual filth isolation procedures.

(3) The results obtained are capable of duplication and are reasonably accurate.

#### ACKNOWLEDGMENTS

The author wishes to express his appreciation to the members of the Central District, U. S. Food and Drug Administration, and to R. L. Vandaveer, in particular, for assistance and invaluable suggestions. The collaborators assisting in this work were E. C. Coulter and H. G. Underwood of Chicago Station; F. J. McNall and I. S. Schurman, Cincinnati Station; L. Jones, Kansas City Station; G. E. Keppel, Minneapolis Station; S. M. Stark and R. L. Vandaveer, New Orleans Station; and C. R. Joiner, St. Louis Station.

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## NOTES

### Determination of Butterfat in Cream and Ice Cream by Dry Extraction with Chloroform\*

Although the Babcock method has been satisfactory for rapid and reasonably accurate butterfat determinations in milk and cream, no simple and uniformly satisfactory method has been developed for the rapid determination of butterfat in ice cream. Analysis of ice cream is made more difficult by the inclusion of cacao products, dextrose, agar, gelatin, egg powder, etc., which increase the tendency to char if the Babcock method is employed, or to emulsify if the Roese-Gottlieb method<sup>1</sup> is used.

Other difficulties encountered in the Roese-Gottlieb method are: (1) the emulsions that frequently form during extraction break up slowly; (2) if separation of the water phase from the organic solvents is not complete, non-fat solids filter through into the weighing dish; (3) butterfat residues from ether and petroleum benzin evaporation tend to creep up the sides of the weighing dish to its edge, which may result in loss of fat; and (4) a Röhrig tube or similar apparatus is required.

The disadvantages of the Roese-Gottlieb method are avoided by dry extraction of butterfat with chloroform. Emulsions do not form, and consequently there is no loss of time or danger of contamination of the extracted fat with non-fat solids; there is no tendency of the fat to creep up the sides of the weighing dish; special laboratory apparatus is not required for the dry-extraction, chloroform method; and mechanical loss of fat is minimized as the entire extraction is carried out in one small flask.

#### METHOD

Warm sample of cream or ice cream to be extracted to room temperature and mix thoroughly. Withdraw a 4 gram portion and place in a 50 ml. glass-stoppered Pyrex Erlenmeyer flask. (Solvent-resistant, synthetic rubber stopper may be used with flask not fitted with a glass stopper.) Add 40 ml. of  $\text{CHCl}_3$ , U.S.P., stopper tightly, and shake vigorously for 2 minutes. Add 10 grams of C.P. anhydrous  $\text{Na}_2\text{CO}_3$ , and immediately shake vigorously for 2 minutes or until adhering film of cream is free of sides of flask. Filter the  $\text{CHCl}_3$ -fat extract through a medium retentive 9 cm. filter paper into a weighed 120 ml. thin Pyrex evaporating dish (filter paper should not

#### *Butter-fat determinations of ice cream and cream by chloroform-dry extraction and Roese-Gottlieb methods*

NO.	SAMPLE	CHLOROFORM-DRY EXTRACTION		ROESE-GOTTLIEB	
		per cent		per cent	
1	Ice Cream	10.34	10.37	10.33	10.05
2		10.22	10.38	10.25	10.35
3		9.91	9.83	9.87	9.82
4		9.19	9.23	9.12	9.11
5		10.10	10.16	10.31	10.28
6		9.34	9.21	9.49	9.59
7		9.61	9.41	9.41	9.43
8		10.68	10.81	10.66	10.68
9		10.08	10.04	9.86	9.84
10		10.87	10.68	10.78	10.85
A	Cream	22.27	22.07	21.90	22.11
B		20.89	20.95	21.15	20.90
C		19.06	19.02	19.04	19.27
D		19.94	19.82	20.05	19.86

\* By George R. Kingsley, Captain, Sn. C., A.U.S., Laboratories, McCloskey General Hospital, Temple, Texas.

<sup>1</sup> *Methods of Analysis, A.O.A.C.*, 1940, 272.



project above the top of the glass funnel). Again extract the residue in the flask for 1 minute with a 20 ml. portion of  $\text{CHCl}_3$ . Repeat the extraction three more times with 20 ml. portions of  $\text{CHCl}_3$  and wash down the sides of the filter. Evaporate the  $\text{CHCl}_3$  slowly on a warm surface and then dry the fat to constant weight (1-1½ hours) in an oven at the temperature of boiling water or on a water bath. Use a blank determination on reagents as a control.

Good agreement was obtained with the Roesse-Gottlieb and chloroform-dry extraction methods, as shown in the table.

### Simple Automatic Device for Controlling Rate of Distillation\*

Whenever it is important or convenient to have rather close, automatic control of rate of distillation it has been found that the following device is useful and easily constructed from materials readily available in the chemical laboratory, and that it controls the rate of steam distillation within 1 or 2 ml. per hour.

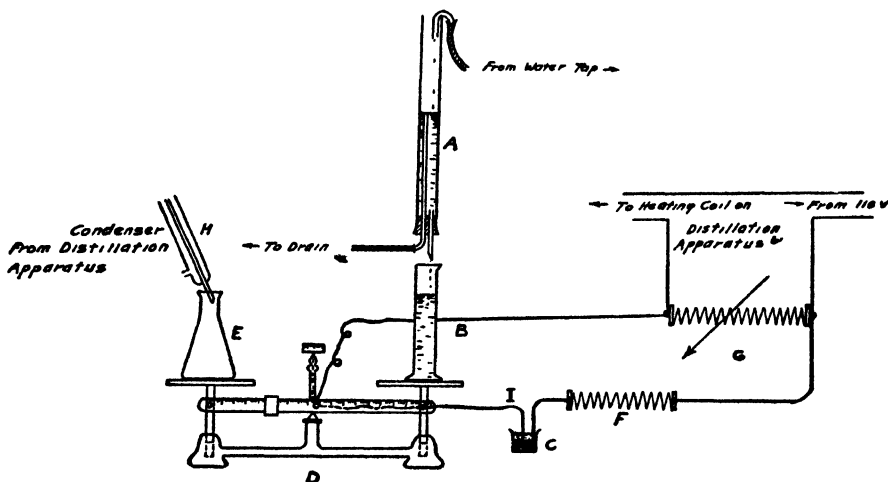
Referring to the diagram the apparatus is described as follows:

Flask (E) and empty graduate (B) are balanced on the platform balance (D), which is equipped with a heavy-gage iron wire (I). This iron wire is connected to a flexible insulated copper wire at the center of the balance and is adjusted to make contact with a pool of mercury in beaker (C). The mercury is covered by distilled water, or amyl acetate, or other non-conducting liquid to condense mercury vapor formed at the contact point. A coil of the same iron wire is inserted under the mercury to transmit the current through resistance (F). A resistance consisting of a 750 watt and a 500 watt heating element in series works satisfactorily. The above circuit is connected across (parallel to) the variable resistance used for rough control of the heating element in the boiling flask.

The device is operated as follows:

Apply full heat to the boiling flask. As soon as distillation starts cut back resistance (G) to a point just under that required for proper distillation rate. Immediately start water running from constant flow device (A) through a drawn-out capillary tube into graduate (B). Previously have the constant flow device adjusted on a *weight basis* to desired rate by raising or lowering the constant level overflow tube in order to compensate for any marked difference in specific gravity of the distillate and the tap water.

If distillation rate is too fast, flask (E) becomes too heavy and breaks contact in (C); if too slow, contact in (E) is made and additional current is supplied to the heating coil at the boiling flask. From then on distillation rate will be maintained exactly as the rate from (A).



\* By W. H. King (State Department of Health, New Orleans, La.)

## SPECIAL PLEA

In the May issue of *This Journal* we ran a conspicuous appeal for back numbers. The response has been gratifying, but the ravages of war have been such that we are constrained to break a long-standing precedent by inserting this brief editorial appeal to go the second mile. That "the burning of the books" has fallen even more heavily on our foreign subscribers than in the first World War is eloquently voiced by a recent request from brave, war-torn China for twenty complete sets of *The Journal*. If we are to fill this and other anticipated orders it will no doubt have to be at the cost of some little sacrifice on the part of our American readers. The numbers needed are listed on the insert in the front of this issue of *The Journal*. We are now imploring you to send us not only all of these back numbers not actually and actively "working," but also those you can spare at the cost of perhaps a few trips a year to the nearest library, or maybe only down the hall a few doors. No cause could be better, and nothing could pay better dividends in the form of Association good will abroad.—THE EDITOR.

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## REPORT ON MONOCHLORACETIC ACID

By JOHN B. WILSON (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

A method for the determination of monochloroacetic acid in beverages was presented by the Referee on Flavors and Non-Alcoholic Beverages at the 1941 meeting of the Association (*This Journal*, 25, 145). In that report a number of determinations of this preservative in orange beverages and in several canned and frozen fruit juices were reported in which the monochloroacetic acid was added to the sample just prior to making the determination.

The following spring an experimental pack of carbonated beverages was prepared for the purpose of ascertaining the effect of storage upon their monochloroacetic acid content. A paper detailing this experimental work has been prepared by the Referee on Flavors and Non-Alcoholic Beverages (*This Journal*, 27, 195).

In the fall of 1942 experimental packs of apple juice and orange juice containing four levels of monochloroacetic acid were prepared under the writer's supervision. Analyses of these samples have also been reported in the same paper.

At the same time an extensive experiment was carried out upon the use of monochloroacetic acid in the manufacture of wine. In this experiment monochloroacetic acid was added to wine at four levels, and the wine was subjected to analysis after various periods of storage. In the course of the latter investigation it was found necessary to modify the procedure somewhat in order that it might give equally good results with wine. The method is the subject of a note published in *This Journal*, 26, 477.

The data obtained so far confirm the conclusion drawn in the original

paper, and they indicate that when monochloroacetic acid is added to carbonated beverages, fruit juices, or wines, some of the acid may be destroyed but that the unchanged acid persists for a considerable period.

During the past year some attention has been given to the qualitative detection of monochloroacetic acid in food products. Mallory and Love of the Alcohol Tax Unit<sup>1</sup> have substantially improved the classic procedure advocated by Chernoff<sup>2</sup> of the U. S. Food and Drug Administration, in which monochloroacetic acid is condensed with anthranilic acid and converted by alkali fusion into indigo. The original procedure caused considerable confusion since it gave good results with pure solutions, but interference was caused by the presence of other ether-soluble substances in food. Mallory and Love have greatly improved the purification of the ether extractives, but in some cases there is still a sufficient quantity of extraneous matter present to make it impossible to follow the color changes in the fusion which indicate its progress and the point at which the melt should be removed from the source of heat. Several analysts, including the writer, have confirmed their contention that it is necessary to dry the mixture thoroughly before making the alkali fusion in order to obtain concordant results.

In collaboration with G. L. Keenan, the Associate Referee investigated the use of barium monochloroacetate to identify this substance microscopically. While it has been possible to identify monochloroacetic acid as barium salt in a large number of cases, here also extraneous matter interferes with proper crystallization of the salt, and negative results do not necessarily mean that monochloroacetic acid is absent.

The procedure used for preparation of the barium monochloroacetate is given in a paper on the identification of monochloroacetic acid as barium monochloroacetate by John B. Wilson and George L. Keenan appearing in this issue.

It is recommended that collaborative study of the methods discussed in this report for the determination of monochloroacetic acid be continued.

## REPORT ON VEGETABLE DRUGS AND THEIR DERIVATIVES

By F. H. WILEY (Food and Drug Administration, Federal  
Security Agency, Washington, D. C.), *Referee*

Due to the pressure of wartime activities, the associate referees and the Referee have had little opportunity to concentrate on A.O.A.C. projects. At least one of the associate referees has joined the armed forces, and a number of the others have been spending full time examining drugs destined for the Army.

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<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 15, 207 (1943).

<sup>2</sup> Personal communication.

Of the thirteen projects assigned, it is recommended that two be discontinued, one be discontinued or reassigned, and the remainder continued. One associate referee has a report for this meeting. Interim reports have been received on six of the projects since the last meeting, but none of them is complete as yet. Six of the associates have made no report. A detailed report on each project is submitted.

*Cinnamyl Ephedrine* (J. Claggett Jones).—The Referee was notified in December 1942 by Mr. Jones that he was going into the Army and would be unable to do any further work on this project. He also gave a review of the work completed thus far. Owing to the difficulties experienced in obtaining satisfactory samples for collaborative study and since the product is relatively unimportant, the Referee recommends that the subject be dropped.

*Chemical Methods for Ergot* (Donald C. Grove).—Dr. Grove reports the development of a method for the separation and estimation of ergometrine. The results by this method have been compared with those obtained by bioassay with very good agreement. The associate referee recommends that the chemical method be subjected to collaborative study. The Referee concurs.

*Theophylline Sodium Salicylate* (M. Harris).—No report was received. The Referee recommends that this subject be dropped entirely or reassigned.

*Prostigmine* (F. J. McNall).—Owing to a misunderstanding, the associate referee worked on Method I outlined in his previous report and submitted a report on this work several months ago. It was recommended at that time that the work be continued and extended to methods that appeared to be more specific.

*Physostigmine in Ointments* (G. M. Johnson).—The associate referee reports that the work has been completed to the point where a collaborative study can be made. The Referee recommends that the subject be continued.

*Quinine Ethyl Carbonate* (H. G. Underwood).—No report was received. The Referee recommends that the subject be continued.

*Theobromine and Phenobarbital* (E. C. Deal).—No report was received. The Referee recommends that the subject be continued.

*Plasmochin* (F. C. Sinton).—No report was received, although the associate referee submitted the material to collaborators some months ago. The Referee recommends that the subject be continued.

*Glucosides and Saponins* (Llewellyn H. Welsh).—In a letter to Dr. Skinner, the associate referee indicated that most of the methods in the literature were of the bioassay type and he did not feel qualified to pursue this study. The Referee recommends that the subject be dropped.

*Strychnine in Pills* (H. C. Lythgoe).—No report was received. The Referee recommends that the subject be continued.

*Phenothiazine* (V. W. Stewart).—No report was received. The Referee recommends that the subject be continued.

*Quinine and Strychnine* (R. L. Herd).—No report was received. The Referee recommends that the subject be continued.

*Polarigraph Methods* (S. Reznek).—No report was received. The Referee recommends that the subject be continued.

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## REPORT ON THE ASSAY OF ERGOT

By D. C. GROVE (Food and Drug Administration,  
Federal Security Agency, Washington, D. C.),  
*Associate Referee*

The chemical assay of ergot is primarily a problem of the separation of the two water-soluble alkaloids—ergometrine and ergometrinine. These two alkaloids are isomers showing very similar chemical properties, but differing in that ergometrine is physiologically active, while ergometrinine is inert. The problem was complicated by the fact that ergometrine and ergometrinine differ in their solubilities in quantity but not in quality.

A process has been devised whereby ergometrine can be separated from ergometrinine by a series of extractions of the ether solution of the alkaloids with water. The quantitative separation of these alkaloids varies with the number of separatory funnels used, the number of washings passed through the series of separatory funnels, and the relative volumes of the solvents used. The calculation of the proper conditions for the separation of any two ingredients of a solution based on their distribution coefficients between the solvents is a very laborious procedure. A graphic method for determining these conditions was developed by Knudsen and Grove.<sup>1</sup>

The results obtained by the chemical assay have been compared with the results obtained by an improved bioassay technic. This comparison indicates that the chemical separation of these two alkaloids is practically complete and that the results obtained by the chemical procedure are in good agreement with those of the bioassay. The details of the chemical assay and the comparison of the results by the chemical and bioassay procedures will be published in the near future. A collaborative study of the chemical procedure has been delayed owing to the pressure of war activities. The Associate Referee recommends that the subject be continued and that a collaborative study be made when this work can be satisfactorily arranged.

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<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 14, 556 (1942).

## REPORT ON PHENOTHIAZINE

By VINCENT E. STEWART (Food and Drug Laboratory,  
Florida Department of Agriculture, Tallahassee,  
Florida), *Associate Referee*

Phenothiazine is rapidly gaining favor as an anthelmintic for veterinary medicine. It is sold in powder form, to be mixed with the feed; in tablets, with various excipients; in tablets, with one or more other drugs; and in suspensions. This report considers only the commercial powder and the tablets not containing other drugs. Methods for the determination of the drug in the other commercial forms will be discussed in a subsequent report.

A method for the determination of phenothiazine in the commercial powder is included in "The National Formulary."<sup>1</sup> This is a colorimetric determination based upon the conversion of phenothiazine to a highly colored red compound, believed to be 3, 9-dihydroxyphenazothionium bromide,<sup>2</sup> by means of bromine water. Cupples<sup>3</sup> utilized a modification of this method for the determination of small amounts of phenothiazine as found in the spray residues of insecticides.

Overholser and Yoe<sup>4</sup> found that palladous chloride will react with phenothiazine to form a dark blue color or precipitate which can be used in the colorimetric determination of phenothiazine in spray residues. They reported that the solutions are not sufficiently stable to be used extensively in transmittancy measurements although a limited application is possible.

Harris and Kerl<sup>5</sup> used an acetone extraction for the determination of phenothiazine in preparations containing no other acetone-soluble substances and reported satisfactory results. The method is applicable to boluses, tablets, granulations, and suspensions in which phenothiazine is the only acetone-soluble ingredient.

## EXPERIMENTAL

This investigation was confined to the acetone (anhydrous) extraction method of Harris and Kerl and the colorimetric method specifying bromine water as the reagent, since these are the only methods reported in the literature that appear to be practicable. The procedure of Harris and Kerl was followed except that a Soxhlet extractor was used instead of an A. S. T. M. extractor. The rapid Goldfisch extractor<sup>6</sup> was also found to be equally suitable. The authors of this method state that the extraction should be continued for about 8 hours. However, extractions of highly purified phenothiazine by means of a Soxhlet extractor siphoning at the

<sup>1</sup> VII, 322-3 (1942).

<sup>2</sup> Eddy, C. W., and DeEds, Floyd, *Food Research*, 2, 305-9 (1937).

<sup>3</sup> Cupples, H. L., *Ind. Eng. Chem., Anal. Ed.*, 14, 53 (1942).

<sup>4</sup> Overholser, L. G. and Yoe, J. H., *Ind. Eng. Chem., Anal. Ed.*, 14, 646-7 (1942).

<sup>5</sup> Harris, L. E. and Kerl, E. B., *J. Am. Pharm. Assoc.*, 31, 47-9 (1942).

<sup>6</sup> Walker, L. S., *This Journal*, 17, 189 (1934).

TABLE 1.—Phenothiazine found (%) in 6 samples

SAMPLES	ACETONE EXTRACTION					ELECTROPHOTOMETER					DUBOSCQ COLORIMETER				
	1	2	3	AV.	AV. DEV. $\pm$	1	2	3	AV.	AV. DEV. $\pm$	1	2	3	AV.	AV. DEV. $\pm$
1. Commercial phenothiazine powder	101.7	101.4	101.0	101.4	0.2	98.8	98.8	98.8	98.8	0.0	95.2	94.2	93.7	94.4	0.6
2. Starch and phenothiazine	72.19	71.53	72.02	71.91	0.26	69.5	70.2	70.0	69.9	0.3	70.2	72.8	71.6	71.5	0.9
3. Lactose and phenothiazine	82.75	82.91	82.91	82.86	0.07	81.4	81.3	81.3	81.3	0.0	77.4	78.8	78.5	78.2	0.6
4. Talc and phenothiazine	76.23	75.76	75.98	75.99	0.16	75.0	75.0	75.6	75.2	0.3	71.8	73.1	72.2	72.4	0.5
5. Commercial tablets phenothiazine—Mfg. A.	79.00	79.02	79.23	79.08	0.10	75.6	74.8	75.0	75.1	0.3	76.7	75.9	75.1	75.9	0.5
6. Commercial tablets phenothiazine—Mfg. B.	91.94	92.36	91.62	91.97	0.26	88.0	87.1	87.3	87.5	0.4	87.1	87.4	86.4	87.0	0.4
Av. of Av. Deviations					0.18					0.2					0.6

rate of about eleven times per hour gave 100 percent recovery by the end of the first hour. This indicates that the prolonged extraction time is unnecessary.

The Associate Referee prepared solutions for the colorimetric determinations according to the procedure of the National Formulary, but observed the warning of Cupples<sup>3</sup> that the saturated bromine water must be added rapidly in order to form the normal red color rather than an off-colored solution with a purple hue giving abnormal results. These solutions were examined in a visual (Duboscq) colorimeter and in a Fisher electrophotometer, a 10 mm. cell without filters being used. The solutions were found to be quite stable, and even after standing for several weeks in the dark there was little change in the electrophotometer reading. The slight increase in reading (increased absorption) observed may possibly be accounted for by evaporation loss of solvent, although the solutions were held in glass-stoppered bottles.

Samples examined were commercial phenothiazine powder, two brands of phenothiazine tablets purchased through retail channels, and mixtures (approximately 1+3) of commercial phenothiazine with the common excipients, lactose, talc, and starch. In the preparation of the mixtures with these excipients some difficulty was experienced in obtaining a homogeneous powder. The method of mixing involved a considerable loss of material so that the final product was not necessarily a 1+3 mixture.

Control solutions for the colorimetric determinations were prepared from highly purified phenothiazine. This was obtained by recrystallizing commercial phenothiazine from toluene with the aid of activated charcoal, as recommended by "The National Formulary."<sup>1</sup> The recrystallization process was repeated, and the product was dried in the oven at 100° and then in a vacuum desiccator.

Results of the analyses are reported in Table 1. In all instances the acetone extraction method gave considerably higher results than did either of the colorimetric methods. Also, commercial phenothiazine powder of a dark green color which obviously contained an appreciable amount of impurities consistently assayed greater than 100 percent by the extraction method. This agrees with the data of Harris and Kerl, which show greater than 100 percent recovery. Acetone extraction of highly purified phenothiazine, however, consistently resulted in almost exactly 100 percent recovery. Therefore, it would appear that the impurities in commercial phenothiazine gain weight in the course of the extraction process (e.g., by solvation, oxidation). The exact cause of this increase has not been determined.

Analyses made with the electrophotometer generally gave higher results than those made with the visual colorimeter although there are two exceptions in the six samples investigated. Also, the average of the



average deviations was greater for the visual colorimetric method than for either of the other two methods.

### CONCLUSIONS

(1) The acetone extraction method is applicable only to products containing no acetone-soluble substances other than phenothiazine. Even with commercial phenothiazine known to contain only a limited amount of impurities this method yields greater than 100 percent recovery so that results are likely to be higher than the true value.

(2) The electrophotometric method of measuring the color developed by phenothiazine with bromine water includes obvious inaccuracies. The amount of sample weighed (0.1 gram of phenothiazine) is so small that routine methods of weighing may introduce an error. The aliquot (5 ml.) of the resulting solution (200 ml.) is likewise so small that extreme care must be used in pipetting.

(3) The visual colorimetric method, employing the same solutions as the electrophotometric method, is open to the same criticisms plus the added inaccuracies inherent in the visual method (*i.e.*, varying degrees of ability of analysts to distinguish colors, visual fatigue, etc.).

(4) Of the three methods investigated the determination of the color developed by phenothiazine with bromine water by means of the electrophotometer appears to be the most satisfactory. Results of analyses made by this method are more nearly in accord with the true values than are those of the other methods, the average deviation is not too excessive, and the analysis can be conducted as rapidly by the electrophotometric method as by either of the other methods.

### RECOMMENDATIONS

It is recommended—

(1) That the electrophotometric method, using bromine water as the reagent, be submitted to collaborators for further study.

(2) That the applicability of the electrophotometric method to products containing other drugs in addition to phenothiazine be investigated, and if found satisfactory that it likewise be studied by collaborators.

### REPORT ON SYNTHETIC DRUGS

By L. E. WARREN (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

With no meeting of the Association in 1942 the work of the Subsection on Synthetic Drugs has been retarded considerably. For the years 1941–1943 fourteen topics have been considered in this sub-section. These are:

Acetanilid	Hydroxyquinoline sulfate
Atabrine	Methylene blue
Benzedrine (in inhalants)	Metrazol
Bromobarbiturates and thiobarbiturates	Phenolphthalein and bile salts
Cinchophen and neocinchophen	Sedormid
Diethylstilbestrol	Sulfanilamide derivatives
Ethylaminobenzoate	Sodium diphenylhydantoin

In the interval since the last meeting two topics, bromobarbiturates and thiobarbiturates and atabrine have been completed by the associate referees with the recommendation that the methods used be adopted as tentative and that the subjects be dropped. Recommendations are being made that three subjects, diethylstilbestrol, sodium diphenylhydantoin, and cinchophen and neocinchophen, be dropped because in the meantime these drugs have been admitted to the U.S.P. or the N.F., together with methods of assay. Because eight subjects have not been brought to a satisfactory conclusion each is being recommended for continuance. However, two of these (methylene blue and sedormid) have been sufficiently perfected to be ready for collaborative study.

Owing probably to war conditions the number of papers dealing with pharmaceutical research has been considerably reduced. Because so many chemists are now engaged in some phase of war production it is becoming more and more difficult to secure associate referees and collaborators. Presumably this difficulty will increase.

No important new books relating to drug analysis have come to the attention of the Referee during the past two years. A new edition of Nicholl's "Aids to the Analysis of Foods & Drugs" was reviewed this year (*This Journal*, 26, 290). Since this work is intended primarily for English law-enforcing officers it is not of great importance to the members of this section. However, a book was published in 1942 which should be of interest to law-enforcement officials every where. It is entitled "Food and Drug Regulations." The author is Steven Wilson, a Pittsburgh lawyer. This publication is primarily a history of Food and Drug Legislation in this country with explanations of how the laws work. The 23rd edition of the "Dispensary of the United States" is just off the press. It follows the same line as the three previous editions. Since the last edition (1937) a new class of powerful therapeutic agents has come into use, *i.e.* the sulfa drugs. The chemistry and therapeutic properties of these are discussed. The historical sections are of interest to the drug analyst, but the assay methods are of but little value to him as they are copied from other sources without critical comment or review.

"The First Supplement to the Pharmacopoeia of the United States" is about ready for publication. It corrects the method for quinacrine hydrochloride tablets and introduces, among other things, stilbestrol and capsules of stilbestrol, succinyl sulfathiazole, sulfadiazine, sulfaguanidine, and tablets of totaquine.

**Benzedrine.**—Two years ago (*This Journal*, 25, 104) a benzoylation method for the determination of benzedrine was adopted tentatively by the Association. The topic was continued in order to study methods for the determination of benzedrine in inhalants and other pharmaceuticals. Since that time no formal report has been submitted. The Referee recommends that the topic be continued.

**Hydroxyquinoline Sulfate.**—This topic has been assigned for several years but no formal reports of progress have been submitted. The Referee is of the opinion that the subject should either be dropped or reassigned.

**Methylene Blue.**—This topic has been under consideration for several years (*This Journal*, 23, 59; 24, 806; 25, 798; 26, 242). The subject was re-investigated because the iodine method (A.O.A.C.) was claimed to be inaccurate. By the method now described in U.S.P. XII the methylene blue is weighed as perchlorate. The associate referee reports that the perchlorate method is satisfactory for certain mixtures (soft gelatine capsules containing methylene blue, and oils of copaiba, santal, and wintergreen), but that the results from coated tablets were unsatisfactory. He recommends that the topic be continued. The Referee concurs.

**Ethylaminobenzoate.**—This product was assigned two years ago but the associate referee reports that no work has been done. The Referee recommends that the topic be reassigned.

**Metrazol.**—The associate referee reports that no work was done. Since metrazol is an important medicament the Referee recommends that the topic be continued.

**Barbituric Acid Derivatives, Particularly Bromobarbiturates and Thio-barbiturates.**—Two methods were used, each of which gave good results. Both have been published. The associate referee could find no marked advantage of one over the other. He recommended that Method I be adopted as tentative for alphenal, calcium ipral, ortal sodium, pentothal sodium, seconal sodium, sandoptal, thioethamyl sodium, nostal, and pernoston. He also recommended that Method II be adopted as tentative for alphenal, alurate, amytal, dial, evipal, ipral calcium, ipral sodium, neonal, nostal, ortal sodium, pentobarbital sodium, pentothal sodium, phanodorn, pernoston, seconal sodium, and thioethamyl sodium. The Referee concurs.

**Acetanilid.**—The topic was reassigned several years ago because the descriptions of the A.O.A.C. methods were asserted to be hazy and redundant. No work has been done. The associate referee asks to be relieved because of occupation with war-production problems. The Referee recommends that the topic be reassigned.

**Sulfanilamide Derivatives.**—Several of the "sulfa" drugs have been studied by the Association (*This Journal*, 22, 81; 23, 745; 24, 803, 810; 25, 56). The last of these drugs studied was sulfathiazole.

Two years ago the associate referee reported that the nitrite assay method for sulfathiazole gave moderately good results under collaborative

trial (*This Journal*, 25, 791). He recommended that the topic be discontinued because the Pharmacopoeia was about to include the drug and describe the nitrite assay method. The Referee recommended that the topic be continued and that the method be applied to tablets and other preparations of sulfathiazole. The associate referee found that the bromination procedure could not be applied to tablets because of the presence of lactose, starch, stearic acid, etc., which react with bromine.

The topic was reassigned but no report has been submitted.

This year the associate referee reports that no work was done. Because of pressure of other work due to war production the associate referee asked to be relieved of this assignment. Some of the sulfa compounds that have been introduced since the Association last studied these topics are sulfaguanidine, sulfadiazine, and succinyl sulfathiazole. The Referee recommends that the topic be continued and reassigned.

*Phenolphthalein in Presence of Bile Salts.*—Preparations are marketed which contain relatively small quantities of phenolphthalein and large amounts of bile salts. This topic was assigned with the view to developing a method for the determination of phenolphthalein in such mixtures. The associate referee has worked out a method by which he obtained 98.5 per cent recovery, but he considers that it is not sufficiently perfected for collaborative trial. It is recommended that the topic be continued.

*Atabrine (Quinacrine Hydrochloride).*—This product has attained prominence in the treatment of malaria. It has many of the properties of the vegetable alkaloids. The associate referee reported that after the topic had been assigned to him the U.S.P. XII admitted atabrine hydrochloride under the name of "quinacrine hydrochloride" and adopted an assay for the product and also for the tablets of the substance. The associate referee adapted the double shake-out method to the salt, using chloroform-ether (3+2) as solvent in an alkaline medium, and obtained good results.

The associate referee and his collaborators then compared the shake-out method with the U.S.P. XII procedure. The results by the two methods are comparable on the salt but they were inferior to those obtained when the methods were applied to tablets. The associate referee recommends that the shake-out method be adopted as tentative as an alternative method. The Referee concurs.

*Sedormid.*—This is a new topic although assigned in 1941. The associate referee has worked out a method by which the substance is decomposed in acid solution, HCl (1+1), to form urea. By the action of urease the urea is converted into ammonia. The method gave good results on the pure chemical but unsatisfactory results on tablets. Later the acidity was increased, HCl(3+2), and the results were better. By extracting the tablets with chloroform the sedormid was obtained in a sufficiently pure state to apply the hydrolysis-urease method. Recoveries of 99.5 per cent were obtained. The method was completed to the stage of collaborative

trials. The associate referee recommended that the topic be continued. The Referee concurs.

*Diethylstilbestrol*.—This synthetic has come into therapeutic use because of its estrogenic properties. It is marketed in the form of ampules, capsules, and suppositories. It was assigned two years ago, but no progress report has been submitted. It is recommended that the subject be dropped because the drug and capsules of it have been admitted to the "Supplement of the Pharmacopoeia of the United States."

*Cinchophen and Neocinchophen*.—This topic was assigned two years ago. No report has been received from the associate referee.

Assays for both cinchophen and cinchophen tablets are described in N.F. VII, and an assay for neocinchophen tablets is described in the U.S.P. XII. In view of these facts the Referee recommends that the topic be placed in abeyance.

*Sodium Diphenylhydantoin*.—This compound was assigned two years ago. The associate referee reported that in the meantime the product, together with an assay, has been admitted to "U. S. Pharmacopoeia XII," p. 153. He recommended, therefore, that the subject be dropped. The Referee concurs.

*Terpin Hydrate in Elixirs and Terpin Hydrate and Codeine in Elixirs*.—Three associate referees have worked on different phases of this topic (*This Journal*, 11, 358; 15, 415; and 23, 757). Comments by various drug analysts indicate that of the two methods for determining terpin hydrate in elixirs, *Methods of Analysis*, A.O.A.C., 1940, 579, 63 and 65(a) and (b), 65(a) is superior. The two later associate referees recommended that the tentative method for terpin hydrate in elixirs (*Ibid.*, 63) be deleted. They further recommended that the tentative method for terpin hydrate and codeine (*Ibid.*, 65(a) and (b)) be advanced to "official, first action." The Referee concurs.

Methods for the substances named below have been recorded as "official, first action" for several years without notable criticisms. It is recommended that their status be advanced from "official, first action"\* to "official, final action."

Monobromated camphor, Method II, *J. Ind. Eng. Chem.*, 14, 24 (1922); *Methods of Analysis*, A.O.A.C., 1940, 576.

Pilocarpine hydrochloride, *This Journal*, 12, 54; *Methods of Analysis*, A.O.A.C., 1940, 589.

Calomel in calomel ointment, *This Journal*, 23, 59; *Methods of Analysis*, A.O.A.C., 1940, 620.

Hypophosphites in sirup, *This Journal*, 23, 59; *Methods of Analysis*, A.O.A.C., 1940, 617.

Salicylic acid in presence of other phenols, *This Journal*, 23, 59; *Methods of Analysis*, A.O.A.C., 1940, 572.

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\* Methods for several of these products were advanced from the status of tentative to that of official in 1940 (*This Journal*, 23, 59).

Tetrachlorethylene in mixtures, *This Journal*, 17, 78; 23, 59; *Methods of Analysis*, A.O.A.C., 1940, 604.

Methods for the substances named below have been recorded as "tentative" for three years or more without serious criticism. It is recommended that the status of each method be advanced from tentative to that of official, first action.

Acetophenetidin and caffeine, U. S. Dept. Agr. Bur. Chem. Bull. 162, p. 193; *Methods of Analysis*, A.O.A.C., 1940, 565.

Acetylsalicylic acid, acetophenetidin, and caffeine, *This Journal*, 22, 91; *Methods of Analysis*, A.O.A.C., 1940, 570.

Bismuth compounds in tablets, *This Journal*, 15, 84; *Methods of Analysis*, A.O.A.C., 1940, 617.

Calcium gluconate, *This Journal*, 17, 75; *Methods of Analysis*, A.O.A.C., 1940, 617.

Effervescent potassium bromide with caffeine, *This Journal*, 21, 96; *Methods of Analysis*, A.O.A.C., 1940, 623.

Iodine, *This Journal*, 15, 83; *Methods of Analysis*, A.O.A.C., 1940, 618.

Mandelic acid, *This Journal*, 22, 98; *Methods of Analysis*, A.O.A.C., 1940, 610.

Oil of chenopodium, *This Journal*, 13, 334; 14, 83; *Methods of Analysis*, A.O.A.C., 1940, 625.

Phenolphthalein in chocolate preparations, *This Journal*, 8, 541; *Methods of Analysis*, A.O.A.C., 1940, 613.

Sulfanilamide, *This Journal*, 22, 97; *Methods of Analysis*, A.O.A.C., 1940, 614.

Theophylline, *This Journal*, 20, 82; *Methods of Analysis*, A.O.A.C., 1940, 593.

*Procaine*.—Wells (*This Journal*, 25, 540) states that Method I, official for procaine (*Methods of Analysis*, A.O.A.C., 1940, 590, 97) is not reliable. According to Wells the method requires 2 hours to stand instead of 15 minutes and an excess of bromine of 140 per cent instead of 127 per cent. In view of this serious charge it is recommended that an associate referee be appointed to restudy the subject.

*Carbromal*.—Carbromal (adalin) was official in U.S.P. XI and is now described in N.F. VII. No method of assay is provided in either compendium. The ready solubility of carbromal in chloroform and ether and its relative insolubility in water indicate that in its quantitative analytical properties it might resemble acetophenetidin.

It is recommended that carbromal be studied and that an associate referee be appointed.

*Phenolsulfonphthalein*.—This drug was described in U.S.P. XI, and is now described in U.S.P. XII, but no assay is given in either publication. It contains about 9.05 per cent of sulfur. The Association has provided no assay for the drug.

It is recommended that phenolsulfonphthalein be studied, and that an associate referee be appointed.

*Sodium Sulfobromophthalein*.—This compound is described in Pharmacopoeia XII, but no assay is provided. The product contains about 38.1 per cent of bromine and 7.653 per cent of sulfur.

It is recommended that sodium sulfobromophthalein be studied and than an associate referee be appointed.

*Propadrine Hydrochloride*.—This is one of the newer synthetics that has ephedrine-like properties. It is also used in the treatment of obesity. It is marketed in capsules, elixirs, jellies, and solutions. The product is described in N.N.R. It is there assayed by determinations of nitrogen and chloride. It is recommended that propadrine hydrochloride be studied.

## REPORT ON BARBITURIC ACID DERIVATIVES

By L. E. WARREN (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Three years ago an associate referee was appointed to ascertain whether the official method for barbital and phenobarbital is applicable to other barbituric acid derivatives used in medicine. Considerable collaborative work was done, and, as a result of the associate referee's report, the official method with slight modifications was adopted as tentative for eight of the more commonly used barbiturates (*This Journal*, 25, 56). These are alurate, amytal, dial, evipal, ipral sodium, neonal, pentobarbital sodium, and phanodorn.

Two years ago the Association decided to continue the work and directed the Associate Referee to apply the modified method to certain other barbiturates not included in the first study, particularly the bromobarbiturates and thiobarbiturates, with the view to ascertaining whether the method already adopted is applicable. No collaborative work was to be conducted, except in doubtful cases.

In the year that followed the following substances were studied by the associate referee: Alphenal (prophenal) powder and capsules, calcium ipral (powder and tablets), nostal powder, ortal sodium, pentothal sodium, pernoston, seconal sodium (capsules), sandoptal, and thioethamyl sodium. The adopted method was applied to market preparations of the barbiturates under consideration, to tablets and capsule contents, and to mixtures prepared to simulate tablets and capsule contents.

The Associate Referee also investigated whether chloroform alone rather than a mixture of chloroform and ether could be used as solvent in the assay of the substances studied. The findings warrant the conclusion that chloroform may be used in the assay of alphenal, calcium ipral, ortal sodium, pentothal sodium, seconal sodium, sandoptal, and thioethamyl sodium. For nostal and pernoston, because of their relatively low solubility in chloroform (800 and 325, respectively), a mixed solvent, although by no means necessary, might be employed with advantage.

Two methods were used on all of the samples tested. Each gave good results. The Associate Referee could find no marked advantage in one over

the other. He recommends, therefore, that Method I be adopted as tentative for alphenal, calcium ipral, ortal sodium, pentothal sodium, seconal sodium, sandoptal, thioethamyl sodium, nostal, and pernoston. He also recommends that Method II be adopted as tentative for alphenal, alurate, amytal, dial, evipal, ipral calcium, ipral sodium, neonal, nostal, ortal sodium, pentobarbital sodium, pentothal sodium, phanodorn, pernoston, seconal sodium, and thioethamyl sodium.

## REPORT ON PHENOLPHTHALEIN IN PRESENCE OF BILE SALTS

By RUPERT HYATT (U. S. Food and Drug Administration,  
Cincinnati, Ohio), *Associate Referee*

Phenolphthalein is present in various combinations in a number of pills and tablets. The tentative method for phenolphthalein in chocolate preparations (*Methods of Analysis*, A.O.A.C., 1940, 613) has been found satisfactory unless bile salts are present. In such instances the iodine precipitate is not normal in appearance, and varying results are obtained.

At the request of Subcommittee B (*This Journal*, 26, 11) a study was undertaken to find a method suitable for the determination of phenolphthalein in the presence of bile salts.

An extraction method was tried in which the powdered material was suspended in water and extracted with chloroform-ether (3+1). The solvent was evaporated, and the residue was treated as directed in the method for phenolphthalein in chocolate preparations. The final iodo-phenolphthalein coagulated when warmed on the steam bath but did not form a gum as it did when contaminated with bile salts.

On a few samples this procedure gave results that apparently were satisfactory. One sample was labeled to contain:

	<i>grams</i>
Powdered Bile Salts.....	1½
Sodium Succinate.....	1½
Sodium Salicylate.....	½
Phenolphthalein U.S.P.....	½
Pow'd Extract Cascara U.S.P.....	½

This method, however, has obvious limitations. If a mixture is alkaline enough to hold back phenolphthalein, salicylic acid would be liberated on acidifying it.

The procedure was then modified to include a wash with sodium bicarbonate solution.\* Most of the succinic acid, if present, will remain in the aqueous solution in the first extraction. The remainder of the succinic acid and the salicylic acid, if present, together with a considerable part of

\* Suggested by L. E. Warren (private communication).



any cascara extractives and bile salts, will be extracted by the sodium bicarbonate solution and thus removed from interference.

Four modifications of the extraction and iodine precipitation method were tried on an old sample with the following results: (1) 3.22 per cent, (2) 3.17 per cent, (3) 3.11 per cent, (4) 3.14 per cent.

The details of the method follow:

#### PHENOLPHTHALEIN IN PRESENCE OF BILE SALTS

*Method 1.*—Suspend the powdered material in water, acidify with dilute HCl, and extract with  $\text{CHCl}_3$ -ether (3+1). Pass each portion of solvent through two separators, each of which contains 10 ml. of freshly prepared 4%  $\text{NaHCO}_3$  solution. Filter the solvent, evaporate to dryness, and proceed as directed in *Methods of Analysis*, A.O.A.C., 1940, 613, 162-164.

*Method 2.*—Proceed as directed in Method 1. Then take up the residue in solvent and alkali, acidify, and re-extract.

*Method 3.*—Extract without acidifying. Pass each portion of solvent through three separators, each of which contains a few ml. of dilute NaOH solution. Discard solvent. Combine the NaOH solutions, acidify, and extract with ether.

*Method 4.*—Proceed as directed in Method 1 except to use washed solvent and not to acidify the sample.

From these experiments it does not appear to be necessary to take phenolphthalein into sodium hydroxide solution (Methods 3 and 4), to wash the solvent, or (in this particular product) to acidify the sample for the extraction.

The following mixture was then prepared and examined by Method 1:

	grains
Sodium Taurocholate (pure).....	5
Sodium Glycocholate (pure).....	5
Phenolphthalein (not dried or assayed).....	1
Phenolphthalein obtained (0.5 gram sample)....	0.0447
Theoretical.....	0.0454
Shortage (moisture or loss in determination).....	0.0007 (1.5%)

Experiments made by the Associate Referee indicate that phenolphthalein may be determined in the presence of bile salts by extraction from aqueous solution (acidifying if necessary), washing the solvent with fresh sodium bicarbonate solution, and proceeding as directed under Phenolphthalein in Chocolate Preparations (*loc. cit.*). It was observed that ordinarily it is not necessary to filter the ice-cold mixture with the bell jar arrangement.

#### REPORT ON QUINACRINE HYDROCHLORIDE (ATABRINE)

By HAROLD C. HEIM (Food and Drug Administration, Federal Security Agency, San Francisco, Calif.), *Associate Referee*

The twelfth edition of the United States Pharmacopoeia describes quinacrine hydrochloride (atabrine) and quinacrine hydrochloride tablets and

also includes assay methods for both products. The problem of devising a method for determining quinacrine hydrochloride was assigned before the compound became official, and it was decided to continue the work in an attempt to devise an alternative method.

Several colorimetric methods for the determination of quinacrine hydrochloride are in the literature, but these methods are designed primarily for minute amounts in body fluids and therefore were thought inapplicable to this problem.

Chemically, quinacrine hydrochloride resembles the alkaloids, yielding precipitates with the usual alkaloidal reagents and forming salts with the mineral acids. From solutions of its salts the free base is precipitated by ammonia or fixed alkalies. The compound is official as the hydrated dihydrochloride. It is soluble in water and alcohol but is insoluble in ether and chloroform. The free base is insoluble in water but soluble in ether and chloroform. Both the free base and its anhydrous salts are hygroscopic.

Because of the solubility characteristics of quinacrine hydrochloride, it was decided to attempt an extraction method whereby the free base is precipitated by ammonia and extracted with a chloroform-ether mixture. The solvent is evaporated, and finally the free base is dried and weighed. Results in reasonable agreement with the theoretical were obtained with this method both with pure quinacrine hydrochloride and also with a simulated tablet mixture consisting of quinacrine hydrochloride, talc, and starch. The method in Pharmacopoeia XII was also applied. It yielded results that closely approximated those obtained by the extraction method, but it proved to be somewhat cumbersome when applied to tablet mixtures.

Samples were sent to collaborators with instructions for assay by both methods. The directions for the extraction method were as follows:

#### EXTRACTION

*Quinacrine Hydrochloride.*—Transfer approximately 0.2 gram accurately weighed, to a separator with the aid of about 30 ml. of water. Add 5 ml. of  $\text{NH}_4\text{OH}$  T.S., shake well, and extract with 20, 20, 10, 10, and 10 ml. portions of a solvent consisting of chloroform 3—ether 2. Combine the solvent portions in a second separator, wash with 10 ml. of water, and filter through cotton into a tared weighing bottle. Evaporate on steam bath with a gentle current of air, dry at  $100^\circ\text{C}.$ , cover tightly, cool, and weigh.

Report percentage of free base and also report as quinacrine hydrochloride U.S.P. XII ( $\text{C}_{21}\text{H}_{19}\text{ClN}_3 \cdot 2\text{HCl} \cdot 2\text{H}_2\text{O}$ ), using the factor 1.2724.

*Quinacrine Hydrochloride in Tablets.*—Weigh approximately 0.35 gram of the finely pulverized material, accurately weighed, into a separator with the aid of about 30 ml. of water. Add an amount of stearic acid equal to approximately 5% of the weight of the sample taken and follow with 2-3 drops of HCl. Extract three times with 15 ml. portions of a solvent (chloroform-ether, 3+2) and collect the solvent in a second separator. Wash the combined solvent layers once with 20 ml. of water and combine the aqueous layer with the contents of the first separator. Discard the solvent layer.

To the combined aqueous layers add 5 ml. of  $\text{NH}_4\text{OH}$  T.S., shake well, and extract with 20, 20, 10, 10, and 10 ml. portions of the solvent. Combine the solvent by filtering each through a small pledget of cotton into a second separator and proceed as directed in the method for the assay of *Quinacrine Hydrochloride*, beginning "wash with 10 ml. of water. . . ."



## IDENTIFICATION

Dissolve the free base in the weighing bottle in ether and add a slight excess of a solution of  $\text{HCl}$  in alcohol (1+10). Stir, and filter with suction. Wash with a little alcohol and ether, dry, and determine the melting point.

Results obtained by both the U.S.P. XII and the extraction methods when applied to a sample of U.S.P. XII quinacrine hydrochloride are listed in Table 1. Table 2 shows results obtained by both methods as applied to a simulated tablet mixture.

TABLE 1.—Results on quinacrine hydrochloride (per cent)

ANALYST	EXTRACTION METHOD			U.S.P. XII METHOD		
	FOUND	THEORY	RECOVERY	FOUND	THEORY	RECOVERY
O. H. Miller	78.75	78.586	100.20	78.62	78.586	100.04
	78.73		100.18			
Neulon Deahl	79.08	78.586	100.63	77.20	78.586	98.23
	79.10		100.65			
H. C. Heim	78.64	78.586	100.06	78.77	78.586	100.23
	78.73		100.18			

TABLE 2.—Results on simulated tablet mixture containing quinacrine hydrochloride (per cent)

ANALYST	EXTRACTION METHOD			U.S.P. XII METHOD			M.P. OF REGENERATED B HCl
	FOUND	THEORY	RECOVERY	FOUND	THEORY	RECOVERY	
O. H. Miller	34.91	34.759	100.43	34.95	34.759	100.55	°C 247–253 (decomp.)
	35.10		100.98				
Neulon Deahl	34.24	34.759	98.507	33.17	34.759	95.429	247–248 (decomp.)
	35.11		101.01				
H. C. Heim	35.08	34.759	100.92	34.04	34.759	97.934	247–252 (decomp.)
	34.91		100.43				

## SUPPLEMENTARY REPORT ON ATABRINE

Atabrine (quinacrine hydrochloride) was assigned for study two years ago. In the interval the substance was admitted to U.S.P. XII, and a

method for the assay of the substance was introduced. The Pharmacopoeia also provided a method for the assay of the tablets. The U.S.P. method for tablets was found unsatisfactory, and a modification is being prepared for the first supplement to the Pharmacopoeia. A method differing considerably from that in the Pharmacopoeia was recommended for adoption by the Associate Referee, but this was not done. Almost at the close of the meeting there was mentioned a method for the assay of the tablets which was in use by the Food and Drug Administration in regulatory work and by manufacturers in control. This method is much simpler than that given in the Pharmacopoeia, and in the absence of interfering substances is believed to be as accurate. It is recommended that the Associate Referee be directed to study the short method. The details of this method follow:

#### ATABRINE

##### *Short Method*

Weigh 50 tablets for average weight. Reduce 20 tablets to a fine powder and transfer about 0.500 gram accurately weighed to a 100 ml. volumetric flask. Add 45 ml. of sodium acetate-acetic acid mixture.\* Shake thoroughly to dissolve quinaerine hydrochloride. Allow to settle and add 50 ml. of 0.1 *N*  $K_2Cr_2O_7$ . Fill to mark with distilled water and mix well. Filter through a dry filter, rejecting the first 15 ml. of the filtrate. Measure 50 ml. of the subsequent filtrate into a glass-stoppered flask. Add 80 ml. of dilute HCl† and 20 ml. of KI, U.S.P. test solution. Stopper the flask and mix by gentle swirling. Allow to stand for 5 minutes and titrate the liberated iodine with 0.1 *N*  $Na_2S_2O_3$ , adding starch T.S. when the end point is neared. 1 ml. of 0.5 *N*  $K_2Cr_2O_7$  = .008482 gram of  $C_{23}H_{10}ClN_3O \cdot 2HCl \cdot 2H_2O$ .

This procedure has also been found advantageous for assaying the drug as well as the tablets.

In order to standardize the potassium dichromate solution in a manner comparable to the assay, the Associate Referee deviated slightly from the method given in U.S.P. XII, p. 754, under "Method II." The mixture was allowed to stand for only 5 minutes in a dark place before titration of the iodine instead of 10 minutes, since the assays call for standing only 5 minutes. The concentrations of the reagents used were also varied somewhat.

### REPORT ON SEDORMID

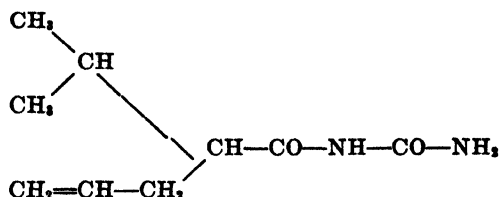
By I. SCHURMAN (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

In accordance with the recommendation of Subcommittee B study of sedormid was undertaken with a view to developing a method for its assay, especially in tablets. It was also considered to be desirable to include an identity test and a test for the presence of barbiturates.

\* Acetate-acetic acid mixture.—Sodium acetate, 2.5 g.; acetic acid, glacial, 1 ml.; water, q.s., 45 ml.

† Dilute HCl.—HCl conc., 15 ml.; Dist. water, q.s., 80 ml.

According to the literature sedormid (allyl-isopropylacetyl-carbamide) is a monoureide having the structural formula:



It is a white crystalline powder, soluble in alcohol, ether, acetone, chloroform, and benzol. It melts at 193°-195°C.

Sedormid may be extracted from a tablet mixture and determined quantitatively by evaporation of the solvent. Recoveries of 99.5 per cent were obtained by this method, but since it is not specific further work along this line was discontinued.

If sedormid is hydrolyzed one of the products is urea. By the application of the urease method, the liberated urea is converted into ammonia, which may be determined quantitatively. Application of this principle to the tablet mixture gave low results. Experiments showed that the hydrolysis of the sedormid was incomplete, owing to the presence of starch. Apparently some of the sedormid material is embedded in the material during granulation and thus resists hydrolysis. This difficulty was overcome by extracting the sedormid from the tablet mixture with chloroform and subjecting the residue to hydrolysis.

In the distillation of the ammonia incessant foaming was encountered. Several anti-foaming agents commonly used, such as paraffin, toluene, caprylic alcohol, diglycol stearate, and carnauba wax were tried, but none was found as effective as the addition of a small amount of a soluble barium or calcium salt. These salts apparently react with the foaming material present in urease to form insoluble compounds.

The pure sedormid powder and the tablets used in this investigation were obtained from a reputable manufacturer. A melting point of 194°-5°C. and a total nitrogen content of 15.00 per cent (theory 15.21 per cent) were obtained on the pure powder.

The proposed method for the assay of sedormid in tablets is as follows:

#### SEDORMID IN TABLETS

Transfer a weighed quantity of the powdered sample containing about 0.3 gram of sedormid to a small beaker. Add  $\text{CHCl}_3$  and mix. Decant the solvent through a filter and wash residue with  $\text{CHCl}_3$  until the material is completely exhausted. Evaporate most of the  $\text{CHCl}_3$  on a steam bath. Transfer residue to a 500 ml. Kjeldahl flask. Evaporate the  $\text{CHCl}_3$  on a steam bath to apparent dryness. Connect flask to suction and remove residual  $\text{CHCl}_3$ . Add 20 ml. of  $\text{HCl}$  (3+2). Place the flask on an asbestos board (with a 2" hole in center), connect with a condenser, and reflux gently for 1 hour. Swirl flask occasionally to wash down any particles adhering to the walls of the flask above the liquid. Cool, and neutralize with  $\text{NaOH}$  (1+1) to

methyl red. Add 50 ml. of water and cool to room temperature. Add two 0.1 gram urease tablets (crushed). Stopper flask and let stand 1 hour. Add 1-2 grams of  $\text{BaCl}_2$ , 150 ml. of water, and 10 ml. of  $\text{NaOH}$  (1+1). Distil the ammonia into a measured amount of standard acid and titrate excess acid with standard alkali, using methyl red indicator. Run a blank and correct results.

1 ml. of 0.1  $N$   $\text{H}_2\text{SO}_4$  = 0.009207 gram of sedormid.

CAUTION: At the beginning of the  $\text{NH}_3$  distillation use a low flame and gradually bring the mixture to a boil. The flame may then be increased without danger of foaming.

The following results were obtained by the proposed method.

(1) *Pure sedormid powder*: 99.06, 98.79, and 98.94 %.

(2) *Sedormid tablets*: Declared 4 grains; found 3.92, 3.94, and 3.96 grains.

(3) *Synthetic sedormid mixture containing 65 % sedormid and 35 % starch*: Found 64.22 and 64.30 %. Average recovery, 98.9 %.

The following identity tests are also presented:

#### IDENTITY TESTS

*Dumont reaction*.—To about 0.1 gram of sedormid in a small porcelain dish add 0.5 ml. of  $\text{HNO}_3$ . Heat carefully over a flame until all nitrous fumes are removed. Evaporate solution to dryness on steam bath. Add 2 drops of  $\text{NH}_4\text{OH}$ . (The residue shows the following color scale: yellow-orange-deep red. After a short time a mauve colored precipitate forms.) Add 5 ml. of  $\text{CHCl}_3$ . (The  $\text{CHCl}_3$  takes on a pink to violet color.)

*Barbiturates*.—Triturate 0.5 gram of sedormid with 5 ml. of water and 3 drops of  $\text{NH}_4\text{OH}$ . Filter, and evaporate the filtrate in a porcelain dish on a water bath. Dissolve residue in 5 ml. of hot water. Add a few drops of  $\text{HgSO}_4$  solution and cool. (The solution does not become turbid.)

Prepare the  $\text{HgSO}_4$  solution by dissolving 7 grams of  $\text{HgSO}_4$  in 25 ml. of water and 4.5 ml. of  $\text{H}_2\text{SO}_4$ , and dilute to 100 ml.

The test is not applicable in the presence of chlorides.

## REPORT ON MISCELLANEOUS DRUGS

By CHRIS K. GLYCART (U. S. Food and Drug Administration,  
Chicago, Ill.), *Referee*

Last year four reports were published without recommendations since the A.O.A.C. meeting for 1942 was omitted. The topics were as follows:

"Report on Microchemical Tests for Alkaloids and Synthetics," by George L. Keenan, *This Journal*, 26, 96.

The study consisted of tests for choline, sulfadiazine, and sodium sulfadiazine. The work was submitted to nine collaborators. In accordance with the favorable results obtained by the collaborators as stated in their comments to the Associate Referee, it is now recommended by him that the test be adopted as tentative. The Referee concurs.

"Quantitative Determination of Quinine by Absorption Spectrophotometry," by Jonas Carol, *Ibid.*, 238.

Over 1000 samples, consisting chiefly of Army medical supplies, were



in the prophylaxis and treatment of malaria makes it desirable to have a rapid method for its determination in tablets and in other forms in which it may be dispensed. The present U.S.P. XII assay, p. 383, requires repeated extractions with ether and is too time-consuming for the analysis of large numbers of samples. The proposed spectrophotometric method is based on the easy solubility of quinacrine hydrochloride in water and the bright yellow color of the resulting solution.

### EXPERIMENTAL

The purity of the quinacrine hydrochloride used in this investigation was established by the U.S.P. XII assay and by determination of the nitrogen content by the Kjeldahl method.<sup>1</sup>

These results are recorded in Table 1.

TABLE 1.—*Analysis of quinacrine hydrochloride used in preparation of absorption data*

SAMPLE	MANUFACTURER	QUINACRINE HYDROCHLORIDE BY U.S.P. XII ASSAY		NITROGEN	
		per cent	per cent	FOUND	THEORY
1	A	99.7	99.1	8.24	8.26
2	B	100.1	99.9	8.27	8.26
3	B	100.0	99.7	8.27	8.26

Solutions containing 4.0 mg. of quinacrine hydrochloride per 100 ml. of 0.1 *N* hydrochloric acid were prepared from each sample, and their absorptions ( $E_{1\text{ cm.}}^{1\text{ per cent}}$ ), relative to 0.1 *N* hydrochloric acid, were measured from 300 to 500  $m\mu$ , a Beckmann quartz spectrophotometer being used. The absorption spectra of the three samples were practically identical, as shown in Table 2 by the absorption data at the principal maxima and minima.

TABLE 2.—*Absorption data*

SAMPLE	$m\mu$	MAXIMA	$m\mu$	MINIMA
		$E_{1\text{ cm.}}^{1\text{ per cent}}$		$E_{1\text{ cm.}}^{1\text{ per cent}}$
1	344	99	320	53
	425	188	365	25
	442	176	437	172
2	344	99	320	56
	425	188	365	28
	442	174	437	173
3	344	98	320	51
	425	188	365	25
	442	173	437	171

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1940, 26.



Figure 1 shows the absorption spectrum of quinacrine hydrochloride from 300 to 500  $m\mu$  and is representative of all three samples.

To test the applicability of the Beer-Lambert law to the solution of quinacrine hydrochloride in 0.1 *N* hydrochloric acid, absorption measurements were made of a series of solutions containing 1.0–5.0 mg. per 100 ml. at 425  $m\mu$ , the wave length of the principal peak absorption. These data

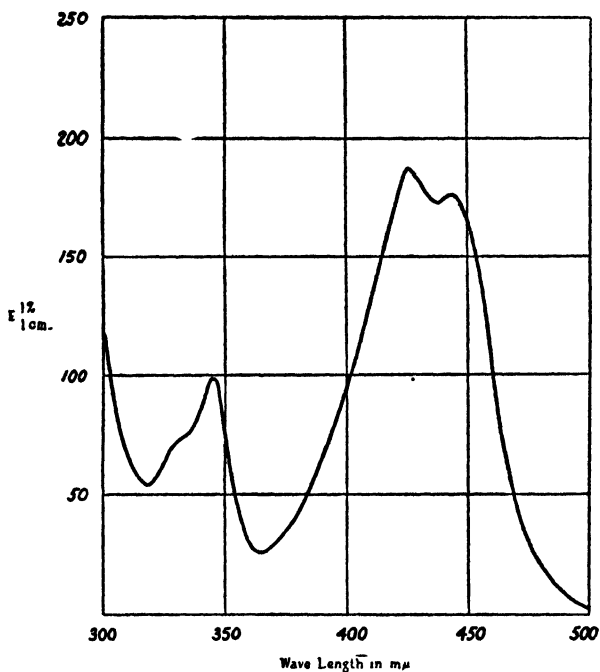


FIG. 1.—THE ABSORPTION SPECTRUM OF QUINACRINE HYDROCHLORIDE IN 0.1 *N* HCl

are recorded in Table 3. The straight line produced in Figure 2 by plotting absorption values against concentration demonstrates that the Beer-Lambert law is satisfied within the limits of experimental error.

TABLE 3.—*Applicability of Beer-Lambert law to solution of quinacrine hydrochloride in 0.1 N HCl*

QUINACRINE HYDROCHLORIDE/ 100 ML. 0.1 <i>N</i> HCl	$E_{1 \text{ cm. } 425 \text{ m}\mu}$
mg.	
1.0	0.188
2.0	0.376
3.0	0.563
4.0	0.751
5.0	0.940

The effect of  $pH$  of solution was determined by measuring the absorption ( $E_{1\text{ cm.}}^{1\text{ per cent}}$ ) relative to the solvent at  $425\text{ m}\mu$  of solutions of quina-crine hydrochloride in normal hydrochloric acid,  $0.1\text{ N}$  hydrochloric acid, distilled water, and  $0.1\text{ N}$  sodium hydroxide. The results in Table 4 show that the absorption is unaffected by  $pH$  changes below 7, but that

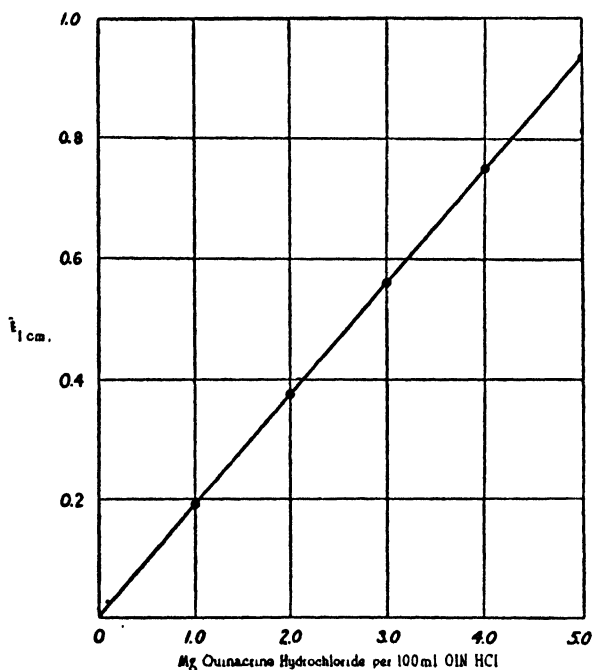


FIG. 2.—PLOT OF  $E$  AGAINST CONCENTRATION  
AT  $425\text{ m}\mu$

alkaline solutions precipitate the base and are unsuited for spectrophotometric analysis.

TABLE 4.—Effect of  $pH$  on absorption of quinacrine hydrochloride solutions

SOLVENT	$E_{1\text{ cm.}}^{1\text{ PER CENT}}$
$N\text{ HCl}$	188
$0.1\text{ N HCl}$	188
Distilled water	188
$0.1\text{ N NaOH}$	Base precipitates

The following method is proposed for the analysis of quinacrine hydrochloride preparations that contain no other water-soluble substance having an absorption band at  $425\text{ m}\mu$ .

## QUINACRINE

## APPARATUS

(a) *Spectrophotometer or photoelectric photometer.*—Having a filter with a peak transmittance at 425 m $\mu$ .

(b) *Matched 1 cm. absorption cells.*

## REAGENTS

(a) *Hydrochloric acid.*—Approximately 0.1 N.

(b) *Quinacrine hydrochloride.*—Determine purity by U.S.P. assay or nitrogen by Kjeldahl method, *Methods of Analysis, A.O.A.C.*, 1940, 26, 21.

(c) *Standard solution.*—2.5 mg. of quinacrine hydrochloride per 100 ml. of 0.1 N HCl.

## DETERMINATION

Accurately weigh or measure a quantity of sample containing about 100 mg. of quinacrine hydrochloride and transfer to 1000 ml. volumetric flask. Add about 100 ml. of the HCl, and heat on steam bath until the quinacrine hydrochloride has dissolved. Cool, and dilute to volume with the HCl.

Filter solution if not perfectly clear. Pipet an aliquot containing 2–3 mg. of quinacrine hydrochloride into 100 ml. volumetric flask and fill to mark with the HCl. Determine the absorption (E) relative to a blank of 0.1 N HCl at 425 m $\mu$ . Also determine the absorption (E) of the standard solution relative to a blank of 0.1 N HCl at 425 m $\mu$ .

$$\text{Mg. of quinacrine hydrochloride in aliquot} = \frac{E \text{ sample}}{E \text{ standard}} \times 2.5.$$

TABLE 5.—Analysis of samples of quinacrine hydrochloride tablets

MANUFACTURER	QUINACRINE HYDROCHLORIDE PER TABLET			ANALYST
	DECLARED	BY SPECTROPHOTOMETER	BY U.S.P. XII ASSAY	
	mg.	mg.	mg.	
a	100	104	103	1
a	100	101	100	1
a	100	104	105	1
a	100	102	102	1
a	100	104	104	1
a	100	100	100	1
b	97.2 (1½ grs.)	95.0	96.0	1
b	100	105	105	1
b	100	99.3	97.6	1
b	100	102	101	1
b	100	96.7	95.8	1
b	100	99.0	99.0	2
b	100	101	102	2
b	100	97.7	95.8	2
b	100	99.5	98.8	2
b	100	96.7	95.0	1
b	100	99.3	99.0	3
b	97.2 (1½ grs.)	95.4	97.1	1

Analyst 1, Jonas Carol; 2, Harold F. O'Keefe; 3, Wm. F. Kunke.

In Table 5 are shown the results of analysis of 18 samples of quinacrine hydrochloride tablets by both the U.S.P. assay and the proposed spectrophotometric method.

#### SUMMARY AND RECOMMENDATIONS

The results of analysis of quinacrine hydrochloride tablets by the U.S.P. XII method and by the proposed spectrophotometric method show good agreement between the two methods. The spectrophotometric method is much more rapid than the U.S.P. XII assay as no extractions are necessary; 25 analyses can easily be made by one analyst in eight hours' working time.

In view of the results obtained it is recommended that the method be adopted as tentative.

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### REPORT ON COSMETICS AND COAL-TAR COLORS

By DAN DAHLE (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

Since the last meeting several changes have taken place among the associate referees. The Associates on Common Ash Constituents, Peroxides in Cosmetics, Hair Dyes and Rinses, Mercury in Cosmetics, Betanaphthol in Hair Lotions, and Nail Cosmetics are no longer doing cosmetic work and the Associates on Lip Make-up, Eye Lotions, and Intermediates in Coal-Tar Colors are in the Armed Forces. In other cases the Associates for one reason or other have been unable to find the time for referee work.

All in all, however, since the last meeting there have been presented five reports on cosmetics and four reports on coal-tar colors. In accordance with the interim provision relative to publication, all but two of these reports have already appeared in *This Journal*. A short summary of such published reports, together with the unpublished recommendations, will be presented.

The Referee makes the following recommendations:\*

(1) That the following methods, now official, first action, be adopted as official, final action:

Sulfides in Depilatory Powders  
*p*-Phenylenediamine in Hair Dyes  
Pure Dye in D&C Orange No. 3  
Pure Dye in D&C Yellow No. 7

(2) That the following methods be adopted as official, first action:

Salicylic Acid in Hair Lotions  
Alizarin in Madder Lake

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\* For report of Subcommittee B and action by the Association, see *This Journal*, 27, 55 (1944).

- (3) That the following methods be adopted as tentative:

Pure Dye in D&C Red Nos. 8 and 31  
Halogens in Halogenated Fluoresceins

- (4) That the following topics be dropped for the duration of the war:

Common Ash Constituents  
Peroxides in Cosmetics  
Betanaphthol in Hair Lotions  
Dentrifrices and Mouth Washes  
Lip Make-up and Rouges  
Eye Lotions

- (5) That the following topics be reassigned:

Mercury Salts in Cosmetics  
Deodorants and Anti-perspirants  
Hair Dyes and Rinses  
Nail Cosmetics  
Intermediates in Coal-Tar Colors

- (6) That the following new topics be studied:

Cosmetic Skin Lotions  
Mixtures of Coal-Tar Colors for Drugs and Cosmetics  
Acetates, Carbonates, Halides, and Sulfates in Certified Colors

- (7) That other topics be continued as listed.

## REPORT ON NAIL COSMETICS

By WILLIAM H. NAYLOR (Long Beach, Calif.), *Associate Referee*

### I. PRELIMINARY SEPARATION OF NON-VOLATILE CONSTITUENTS

Nail enamels are lacquers adapted for use on the nails of the fingers and toes. They usually consist of a solution of a cellulose ester, a plasticizer, and a resin in a solvent, which is a mixture of organic compounds. There are two types of nail lacquer on the market, namely clear and "cream." The cream type contains a suspended pigment which imparts a creamy appearance. The clear type contains no insoluble pigment, but it may have color.

Nitrocellulose is the cellulose compound most commonly used. However, other cellulose esters, as ethyl cellulose, cellulose acetate, etc., have been proposed for use. Camphor is quite commonly present in nail lacquers as a plasticizer, although a wide variety of other compounds may be encountered. The resins are likely to be the synthetic ones, since they are more constant in composition and usually clearer in color.

The insoluble pigment in several of the cream-type enamels is titanium dioxide, frequently so finely divided that it is difficult if not impossible to

effect a separation with a centrifuge. Other types of insoluble materials are used; for example, a mother of pearl effect is said to be achieved by use of small crystal plates of quinin, a substance derived from fish scales.<sup>1</sup>

The choice of solvent is important, for if the evaporation is too rapid the cooling will cause condensation of moisture and ruin the surface. Conversely, too slow evaporation will be undesirable. However, slower drying lacquers are less susceptible to chipping and peeling. The vapor pressure of a solvent at body temperature is a better criterion of its evaporation rate than is its boiling point, since the evaporation rates of substances at a given temperature do not necessarily increase as their boiling points decrease.

The type of nitrocellulose used in lacquers<sup>1</sup> is a mixture of the di- and trinitrated cellulose containing about 12 per cent nitrogen, not the fully nitrated cellulose or gun cotton. It is soluble in esters, ketones, aldehydes, organic acids, and ethers that have an OH group in the molecule. It is also soluble in a mixture of ethyl ether and ethyl alcohol, but not in either of these substances alone. The alcohols and ethers are sometimes called extenders, since they may be used to dilute a solution of nitrocellulose in one of its true solvents. Hydrocarbons are also used as diluents. Here the dilution ratio is finite, and excessive addition of hydrocarbons will cause precipitation of the nitrocellulose. The aromatic compounds have higher dilution ratios, i.e., a larger quantity of toluene may be added before precipitation occurs than is possible with normal hexane. Hydrocarbons are used primarily to reduce the solvent cost.

The investigation of solvents was not undertaken because of the lack of adequate fractionation equipment. The separation of lacquer solvents into their constituents even with the best fractionating column will be fraught with difficulty because of the formation of azeotropes. The solvent can be separated from the solids by ordinary distillation of a large sample to a small volume followed by a steam distillation. This last step will probably carry over some of the plasticizer.

The total non-volatile matter in a lacquer is not effectively determined by mere evaporation because if the lacquer is not spread out in a very thin film, solvent inclusion is likely to occur. Water, petroleum ether, benzene, or chloroform may be added to precipitate the nitrocellulose and other materials. Water will precipitate the resins and nitrocellulose, but the stringy precipitate produced and the relative difficulty of evaporating the water make it by far the least desirable precipitant. Any of the three organic precipitants mentioned may be used in the determination of total solids. A convenient procedure is as follows:

#### METHODS

*Total Solids.*—Place the lacquer in a sample bottle of the Grethen type or some

<sup>1</sup> McDonough, Everett G., "Truth About Cosmetics," p. 137 *et seq.* Drug and Cosmetic Industry, New York (1925).

modification thereof, where evaporation loss is held to a minimum. Run the samples out into platinum dishes containing some of the precipitant and weigh by difference. Place the dishes in a stream of dust-free air with perhaps slight warming and allow to evaporate. When the liquid is almost gone, add more precipitant and break up the precipitate so that there will be less liquid inclusion as the last of the precipitant is removed. Carry the evaporation to completion and determine the increase in weight of the platinum dishes.

*Ash.*—Place the platinum dishes containing the solids in a muffle furnace and heat gradually to 550°C. (The ash will serve to determine inorganic pigments, as for example, titanium dioxide, although for best accuracy a blank should be determined for a clear lacquer of the same composition since nitrocellulose itself leaves an appreciable ash.)

*Separation of Nitrocellulose and Dye from the Other Constituents.*—Weigh 2–3 gram aliquots into 100 ml. beakers half filled with  $\text{CHCl}_3$ . Run the lacquer into the  $\text{CHCl}_3$  dropwise or in a thin stream so that the precipitate will form small pieces. Transfer the precipitate to a weighed, glass Soxhlet thimble (asbestos mat or fritted disc), wash with  $\text{CHCl}_3$ , and collect the filtrate in a crystallizing dish. Extract the precipitate with  $\text{CHCl}_3$  for 3–4 hours in a Soxhlet extractor. Add the extraction  $\text{CHCl}_3$  to the filtrate in the crystallizing dish and evaporate the mixture in an air current. When the odor of  $\text{CHCl}_3$  is gone, take up the residue in  $\text{CHCl}_3$ , filter, and wash into a weighed vessel. Allow the  $\text{CHCl}_3$  to evaporate as before and place the dish with a similar tare in a desiccator before weighing. Cool, weigh, and record as “resins and plasticizers.” (The filtration step is necessary because the solvent contained in the original  $\text{CHCl}_3$  precipitation holds some of the nitrocellulose in solution. When the solvent is removed by evaporation, the nitrocellulose may be precipitated.)

Dissolve the residue from the filtration of the resins and plasticizers through the filter into the original precipitation beaker, using as small a quantity of acetone as possible. Dissolve the nitrocellulose and dye remaining in the beaker and evaporate the acetone to very small volume. Extend the solution with alcohol and again evaporate, then add  $\text{CHCl}_3$ , transfer the precipitate to the Soxhlet thimble, dry, and weigh.

Table 1 gives some typical data obtained by this procedure.

TABLE 1.—*Typical results obtained*

SAMPLE	NITROCELLULOSE AND DYE		RESINS AND PLASTICIZERS		REMARKS
	per cent		per cent		
1	14.31	14.50	12.05	12.05	dark red
2	10.59	10.58	12.17	12.50	colorless overcoating
3	15.95	16.33	14.16	13.98	very light pink
4	10.93	11.28	12.22	11.41	bright red
5	19.21	19.91	5.22	4.75	colorless overcoating
6	13.65	—	13.67	13.42	light red
7	—	—	12.97	13.03	dark red
8	17.30	17.44	13.64	13.90	bright red
9	15.76	15.83	—	—	very dark red
10	16.72	17.36	—	—	colorless overcoating

In all samples investigated, the chloroform extraction method separated the nitrocellulose and the color as a precipitate, with the resins and the plasticizers in the filtrate. If camphor is present in the “resins plus plasticizers” thus separated, the dish containing them must not be heated

or the camphor will be lost. On the other hand, if after the total resins and plasticizers have been weighed, the dish is then heated (preferably in a non-oxidizing atmosphere), the camphor or other volatile plasticizers may be driven off quantitatively. This last point has not been investigated thoroughly, however.

Attempts to separate the nitrocellulose quantitatively from the dyes have not been successful. Regeneration of the cellulose from nitrocellulose by use of ammonia and alkali sulfides was not quantitative. The determination of nitrogen can not serve to evaluate the amount of nitrocellulose, because most of the dyes used contain nitrogen. Partial separation for qualitative purposes may, however, be effected in various ways. Some dye may be removed by treating an ethyl acetate solution with ammonia water. Some of the nitrocellulose is decomposed, and there is considerable trouble with emulsification. Dye may be separated on wool from an acetone-water mixture or on silk from an alcohol medium. Nitrocellulose free from dye may be secured by slowly adding chloroform to an alcohol-ether solution, from which most of the ether has been removed by evaporation, until the first precipitate appears. This precipitate is filtered and washed with a mixture of  $\frac{1}{3}$  alcohol and  $\frac{2}{3}$  chloroform. If the precipitation is stopped in time, the nitrocellulose will contain very little color; if it is carried to completion, it will contain essentially all the color. A similar separation may be carried out by adding water to a solution of the material in glacial acetic acid. When the nitrocellulose is obtained relatively color free, its presence may be confirmed by testing a film of it on a microscope slide with a 1 per cent solution of diphenylamine in concentrated sulfuric acid. The presence of nitro groups is indicated by an intense blue.

The best hope for quantitative estimation of nitrocellulose and dye in a mixture appears to be the use of a spectrophotometric method. If the qualitative determination of the dye can be made from its absorption spectrum then its concentration can be determined from the extinction or optical density at an absorption peak.

The literature on lacquer analysis is rather scarce, but some of the additional references consulted in this work are listed.

It is recommended that the study of this topic be continued.

#### ADDITIONAL REFERENCES

- (1) GARDNER, HENRY A., "Physical and Chemical Examination of Paints, Varnishes, Lacquers, and Colors," 5th ed. (1930). Distributed by Institute of Paint and Varnish Research, Washington, D.C.
  - (2) CONLEY, A. D., *Ind. Eng. Chem.*, 7, 882 (1915).
  - (3) GARDNER, HENRY A., "Examination of Pyroxylin Lacquer Coatings," *Circ. No. 227*, Paint Manufacturers' Assoc., Washington, D. C. (1925).
  - (4) WILSON, SAMUEL P., "Pyroxylin Enamels and Lacquers," p. 194. D. Van Nostrand, New York (1925).
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## REPORT ON BUFFERS AND SOLVENTS IN TITANIUM TRICHLORIDE TITRATION

By O. L. EVENSON (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Samples of D&C Red Nos. 8, 19, and 31 were sent to the following collaborators:

Allied Chemical and Dye Corporation (National Aniline Division)  
 Ansbacher-Siegle Corporation  
 Calco Chemical Company  
 H. Kohnstamm & Company  
 S. S. Forrest, U. S. Food and Drug Administration

Collaborators were asked to determine pure dye and volatile matter by the following methods:

### VOLATILE MATTER

Weigh about 2 grams of sample into a weighing bottle about 1.5 inches in diameter and dry in an air oven at 135°C. for 6 hours or overnight. Cool in a desiccator and weigh. Repeat the heating and weighing at hour intervals until the weight becomes constant ( $\pm 1.0$  mg.). Report the loss in weight as volatile matter.

### PURE DYE IN D&C RED NO. 8 AND D&C RED NO. 31

In a wide-mouthed Erlenmeyer flask dissolve 0.2 gram of the sample in 5 ml. of conc.  $\text{H}_2\text{SO}_4$ . Dilute with 100 ml. of water and add sufficient 30%  $\text{NaOH}$  to leave the solution slightly acid. Add 15 grams of sodium bitartrate and heat to dissolve the buffer. Add approximately 125 ml. of 95% alcohol, heat to boiling, and titrate slowly, especially towards the end, with  $\text{TiCl}_3$  to a yellow end point.

1 ml. of 0.1  $N$   $\text{TiCl}_3$  = 0.00997 gram of D&C Red No. 8; 1 ml. of 0.1  $N$   $\text{TiCl}_3$  = 0.007783 gram of D&C Red No. 31.

### PURE DYE IN D&C RED NO. 19

Dissolve 2.5 grams of color in a 500 ml. volumetric flask. Dilute to the mark, mix, and pipet 100 ml., corresponding to 0.5 gram, into a wide-mouthed Erlenmeyer flask. Add 200 ml. of 95% alcohol and 20 grams of sodium tartrate. Heat, and titrate slowly with standard  $\text{TiCl}_3$ . Keep the solution at the boiling point during the reduction period. When the solution is practically colorless, add about 1 ml. of  $\text{TiCl}_3$  in excess and wash down the side of the flask with a spray of water. When all dye has been completely reduced, remove the flame, and add 25 ml. of conc.  $\text{HCl}$  and about 5 grams of  $\text{NH}_4\text{CNS}$ . Titrate back at once with 0.1  $N$   $\text{Fe}_2(\text{SO}_4)_3$  until a persistent red is obtained. If it appears that the end point has been overstepped, use a few drops of  $\text{TiCl}_3$  to bring it back.

1 ml. of 0.1  $N$   $\text{TiCl}_3$  = 0.02395 gram of D&C Red No. 19.

The results are given in the table in the order the reports were received.

As may be seen from the table, Collaborator 3 reported results well over 100 per cent for the pure dye in all three of these samples. According to his comments he was unable to obtain a definite end point. For D&C Red Nos. 8 and 31 closely agreeing results were obtained by the other collaborators. For D&C Red No. 19 Collaborator 2 also reported results of

COLOR	COLLABORATOR	PURE DYE	VOLATILE MATTER	SUM PURE DYE AND VOLATILE MATTER
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
D&C Red No. 8	1	94.4	6.8	101.2
	2	93.0	—	—
	3	106.5*	6.5	113.0*
	4	93.0	6.9	99.9
	5	92.8	7.1	99.9
	—	—	—	—
	Av.	93.3	6.8	100.3
	Av. dev.	±0.55	±0.17	± 0.57
D&C Red No. 19	1	98.1	1.8	99.9
	2	125.0	1.4	126.4
	3	116.6*	2.3	118.9*
	4	97.5	1.6	99.1
	5	98.5	3.7	102.2
	—	—	—	—
	Av.	104.8	2.16	106.9
	Av. dev.	±10.1	±0.67	±9.7
D&C Red No. 31	1	94.5	0.8	95.3
	2	94.2	—	—
	3	111.7*	0.7	112.4*
	4	93.7	0.8	94.5
	5	96.4	1.0	97.4
	—	—	—	—
	Av.	94.7	0.8	95.7
	Av. dev.	±0.85	±0.07	±1.1

\* Not included in average figures.

more than 100 per cent for pure dye. His comments suggested that this was due to the method as written.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the method for pure dye in D&C Red Nos. 8 and 31 be adopted as tentative.

(2) That the method for D&C Red No. 19 be rewritten and submitted to further collaborative study.

\* For report of Subcommittee B and action by the Association, see *This Journal*, 27, 58 (1944).

## REPORT ON MALT

By CHRISTIAN RASK (Albert Schwill & Company, Chicago, Ill.),  
*Associate Referee*

The present method for the determination of bushelweight is based upon the method described in the "Handbook of Official Grain Standards of the United States" (rev. ed. June 1937), which specifies the use of a quart-sized receptacle.

As these specifications call for uncommonly large samples many variations of this equipment are in use. Of these, the most popular one has a pint-sized receptacle.

Because the samples available for laboratories frequently are even smaller than one pint the American Society of Brewing Chemists appointed a committee headed by S. Jozsa to conduct a study, with the purpose of developing a method that would make possible determinations on small samples.

The method eventually developed determines the bushelweight by measuring the volume of a given weight. This is in contrast to the present method, which determines the weight of a standard volume. However, the same theory has been used in the past by Brauer, Greiner, Streitz, and others. The equipment needed is quite simple and inexpensive. It consists of a 250 ml. Normax cylinder, graduated to meet N.B.S. specifications, and a metal funnel provided with a plunger discharge. The funnel must fit snugly into the graduate and be large enough to accommodate the grain without danger of spilling it when the plunger is raised. The weight of the sample was set at 110 grams.

The determination is carried out as follows:

## BUSHELWEIGHT

Weigh 110 grams of the sample to the nearest 0.1 gram, and pour evenly into a metal funnel provided with a plunger discharge and placed on top of a 250 ml. Normax cylinder graduated to meet N.B.S. specifications. The funnel must fit snugly into the graduate and be sufficiently large to accommodate the grain without danger of spilling when the plunger is raised. Then drop the material into the cylinder by raising the plunger. Do not jar or tap the cylinder during this operation or before reading the volume, and do not read the very upper grain level, as compensation must be made for the ends of the few kernels that protrude. If the grain surface has a slant, repeat the test.

Make the calculation, which is based on the consideration that the Winchester bushel used in this country holds 2150.42 cu. in. or 35.239 ml., as follows:  $X = W/Y$ , where  $X$  represents bu. wt. in lbs.,  $Y$  the volume in Winchester bushels, and  $W$  the weight in lbs. If weight is constant ( $K$ ),  $X = K/Y$  or  $XY = K$ . To ascertain  $K$ , let bushelweight equal unity, then with the constant weight of sample in the test 110 grams—

$$\frac{1 \text{ lb.}}{1 \text{ bu.}} = \frac{453.6 \text{ grams}}{35.239 \text{ ml.}} = \frac{110 \text{ grams}}{8545 \text{ ml.}}$$

Therefore  $K = 8545$  and

$$\text{bushel weight in lbs.} = \frac{8545}{\text{Volume occupied by 110 grams of sample in ml.}}$$

For collaborative work a set of samples consisting of three malts and four barleys was prepared. In order to obtain the actual bushelweights the samples were first tested by the Chicago Branch of the Federal Grain Supervision, U. S. Department of Agriculture. The results on duplicate tests varied less than 0.1 lb. The samples were then circulated among five collaborators, who reported the results shown in the table.

*Collaborative Results*

COLLABORATOR	MALT			BARLEY			
	1	2	3	1	2	3	4
Official U. S. Department of Agriculture							
	38.45	39.55	35.15	48.85	49.40	46.75	42.10
A.S.B.C. proposed, volumetric, with plunger discharge							
1	38.40	40.00	34.50	48.75	49.40	46.50	42.00
2	38.40	39.15	35.15	48.75	48.95	46.55	42.00
3	38.58	39.47	34.74	48.91	49.54	46.44	41.69
4	38.15	39.10	34.85	48.40	49.10	46.55	41.90
5	38.37	39.63	34.75	48.63	48.75	46.25	41.75
Spread:	0.43	0.90	0.65	0.51	0.79	0.30	0.31
Average:	38.38	39.47	34.80	48.69	49.15	46.46	41.87
A.S.B.C. proposed, volumetric, with slide discharge							
1	38.25	40.00	34.25	48.75	49.75	46.50	42.00
2	38.55	39.15	35.00	48.65	49.40	46.90	42.05
3	38.41	39.74	34.95	48.69	49.25	46.44	41.58
4	38.90	39.80	35.85	49.40	49.85	47.05	42.20
5	38.32	39.45	34.81	48.83	48.83	46.19	41.68
Spread:	0.65	0.85	1.60	0.75	1.02	0.86	0.62
Average:	38.49	39.63	34.97	48.66	49.62	46.42	41.90
Present method practised*							
1	39.00	40.25	35.25	49.75	49.75	47.00	42.25
2	38.25	39.25	34.50	48.50	49.25	46.50	42.00
3a	38.60	39.73	35.35	49.40	49.95	47.20	42.30
3b	38.25	39.62	35.25	49.00	49.25	46.63	41.68
4	38.20	39.00	35.20	48.50	49.00	46.50	41.50
5	—	—	—	—	—	—	—
Spread:	0.8	1.25	1.30	1.25	0.95	0.70	0.80
Average:	38.46	39.57	35.11	49.03	49.44	46.77	41.95

\* 1 Modified Brauer Volumetric.

2 Pint-sized bu. wt. tester.

3a Quart-sized bu. wt. tester.

3b Pint-sized bu. wt. tester.

4 Pint-sized bu. wt. tester.

5 Did not report

The results are very satisfactory and demonstrate the soundness of the proposed method. On the basis of these studies it is therefore recommended that the method presented be adopted as an alternative procedure for the determination of bushelweight in malt.

With further reference to the report on caramel and black malts submitted earlier this year, the Associate Referee wishes to point out that these methods have been tested by collaborative studies over a period of three years.

On the basis of these studies it is therefore recommended\* that the methods for the analysis of caramel and black malts printed in *This Journal*, 26, 300-301, be adopted as tentative.

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### REPORT ON DIASTATIC ACTIVITY OF MALT

By ALLAN D. DICKSON (Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture, Agricultural Research Administration, Madison 6, Wis.), *Associate Referee*

The A.O.A.C. adopted as tentative the method for diastatic power of malt of the American Society of Brewing Chemists. It involves a Fehling titration procedure for determination of the reducing power of hydrolyzed starch solution. In 1937 Anderson and Sallans<sup>1</sup> proposed the use of the ferricyanide procedure of Blish and Sandstedt.<sup>2</sup>

In the reports of the Associate Referee on Diastatic Activity of Malt for 1939 and 1942,<sup>3,4</sup> the suggestion was made that the ferricyanide procedure be adopted after collaborative work had been completed by the American Association of Cereal Chemists and the American Society of Brewing Chemists.

In 1938, The Malt Analysis Standardization Committee of the A.A.C.C.<sup>5</sup> carried out a survey collaborative study of methods for diastatic power. In a small number of laboratories, the ferricyanide procedure showed less variation between laboratories than did the A.S.B.C. method. In 1939-40 the same committee conducted a rather comprehensive collaborative study of the ferricyanide method.<sup>6</sup> Although the unexplained variations between laboratories were greater than was considered to be desirable, they were less than those from any other method studied. The A.A.C.C. adopted the ferricyanide procedure as tentative, as well as the method of the A.S.B.C.

The following year a joint collaborative study of these two methods was carried out by the A.S.B.C. and the A.A.C.C., with 16 collaborators

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 27, 64, 82 (1944).

<sup>1</sup> *Can. J. Research*, C15, 70 (1937).

<sup>2</sup> *Cereal Chem.*, 10, 189 (1933).

<sup>3</sup> *This Journal*, 22, 200 (1939).

<sup>4</sup> *Ibid.*, 25, 265 (1942).

<sup>5</sup> *Cereal Chem.*, 16, 353 (1939).

<sup>6</sup> *Ibid.*, 17, 645 (1940).

taking part. Reports of this study were presented to both associations.<sup>7,8</sup> The two methods gave almost identical average values at the four levels of activity, and the variation between laboratories was similar. This study verified the conversion factor from ml. of ferricyanide to degrees Lintner suggested by Anderson and Sallans.<sup>1</sup> The A.S.B.C. adopted the ferricyanide procedure as an alternative standard method for determining the reducing power after diastasis, and both societies recommended the use of the factor 23 in converting from net ml. of ferricyanide to degrees Lintner when the A.S.B.C. method using 200 ml. of starch and a final volume of 250 ml. are employed.

A third collaborative study of the two modifications was carried out jointly by the two societies in 1942. The data in Table 1 of that report<sup>9</sup> indicate further that both procedures give values of the same magnitude.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the ferricyanide modification for the determination of reducing power after diastasis be adopted as tentative and used as an alternative method to the Fehling titration procedure.

(2) That paragraph 48, *Methods of Analysis*, A.O.A.C., 1940, 161, be modified according to the recommendation of Rask<sup>4</sup> by the addition at the end of the second paragraph of the following, "Determine reducing power by I, Fehling's modification, or II, ferricyanide modification. The revised copy for both of these modifications was published in *This Journal*, 27, 82 (1944).

(3) That diastatic power values be reported both as degrees Lintner and maltose equivalent until such time as degrees Lintner can be dropped.

#### REPORT ON HOPS

By FRANK RABAK (Bureau of Plant Industry, Soils, and Agricultural Engineering, A.R.A., U. S. Department of Agriculture, Washington, D. C.), *Associate Referee*

Because of the growing importance, in recent years, of the analysis of hops for the scientific evaluation of their brewing quality, attention has been directed by investigators both here and abroad to the development of satisfactory methods for the quantitative determination of those constituents on which their quality mainly depends. Work on the development of suitable methods was inaugurated by the American Society of

<sup>7</sup> *Cereal Chem.*, 19, 249 (1942).

<sup>8</sup> *Proc. Am. Soc. Brew. Chem.*, 90 (1941).

<sup>9</sup> *Cereal Chem.*, 20, 31 (1943).

\* For report of Subcommittee D and action by the Association, see *This Journal*, 27, 64, 82 (1944).

Brewing Chemists in 1938, when collaborative tests were begun on the Laufer method,<sup>1</sup> which is a modification of the methods of Ford and Tait<sup>2</sup> and of Wöllmer.<sup>3</sup> Later the method of Rabak,<sup>4</sup> which is a modification of the Walker-Hastings method,<sup>5</sup> was included in the collaborative tests. The two methods by Laufer and Rabak, which were found by collaborators to give about the same results, were then combined in order to retain the merits of both. After the incorporation of various suggestions by collaborators to improve the methods, the unified method, comprising both physical and chemical analysis, was furnished to collaborators for use in the collaborative work of 1940. In view of the fairly satisfactory agreement in results reported by collaborators during that year it was recommended by the American Society of Brewing Chemists that the proposed method be adopted subject to slight modification pending further suggestions by collaborators. The complete unified methods, including sampling, physical examination, and chemical analysis, were recommended for adoption and published as tentative methods for the analysis of hops.<sup>6,7</sup> Collaborative tests were continued in 1941, and the 18 participating collaborators were requested to follow carefully the methods of analysis as published, to make comments on the various procedures, to suggest constructive changes, and to report on modifications of the moisture, alpha and gamma (hard) resin determinations previously suggested by certain collaborators. The reports of the collaborators in the final tests indicated that in general the results were in good agreement and that the methods with certain changes in the procedure for the determination of the total soft and alpha resins and minor changes in other procedures were quite satisfactory.

#### *Recommended Changes in Method*

Under **PHYSICAL EXAMINATION (f) Seeds** (*This Journal*, 25, 293) insert in the first sentence of par. 2, after the word "hands," "using a circulatory motion." In the same paragraph after the words "nearest 0.01 gram," p. 294, insert the following sentence: "By means of forceps remove all particles of spindles from the seed in the tared dish before weighing."

Under **CHEMICAL ANALYSIS (PREPARATION OF SAMPLE)**, p. 294, change the third sentence to read: "Pass the hops evenly and slowly through the grinder, taking care to avoid choking the orifices and thus preventing undue heating of the hops."

Under **MOISTURE**, p. 294, change (3) to read, "Drying for 2 hours at a temperature of 103°–104°C." Also in the first sentence of last paragraph, after the words "in a 70 mm. dish," insert the following sentence: "The amount of hops and the dimensions of the dishes used are important for accurate results."

<sup>1</sup> Laufer, S., and Laufer, L., *Am. Brewer*, 71, No. 9, 27 (1938).

<sup>2</sup> Ford, J. S., and Tait, A., *J. Inst. Brewing*, 38, 351 (1932).

<sup>3</sup> Wöllmer, W., *Allgem. Brauer-u. Hopfen Ztg.*, 70, 1531 (1930); *J. Inst. Brewing*, 37, 81 (1931).

<sup>4</sup> Rabak, F., *Communications Master Brewers Assoc. Amer.*, 11, No. 2 (1938).

<sup>5</sup> Walker, T. K. and Hastings, J. J. H., *J. Inst. Brewing*, 39, 509 (1933).

<sup>6</sup> *Proc. Am. Soc. Brew. Chemists*, 4th Ann. Meeting, 1941, pp. 130–139.

<sup>7</sup> *This Journal*, 25, 292 (1942).

Under **DETERMINATION, Soft Resins**, p. 294, in the 2nd sentence in par. 1 change "100 ml." to "about 100 ml." In next to the last sentence in par. 2, p. 295, after the words, "Wash the paper and funnel," insert, "thoroughly until all traces of resins are dissolved and." In par. 3, line 4, p. 295, after the words "side-arm distillation flask," add the words "or Soxhlet extraction flask." Change the sentences immediately following in same paragraph, p. 295, to read as follows: "Evaporate off the solvent by distillation on a water or steam bath at 60°C., using vacuum and driving off the last 5-10 ml. by applying suction to the flask while it is kept immersed in the water or steam bath until constant weight is attained (usually after 5 minutes). Weigh the flask after it has stood near the balance for 30 minutes." Change the last paragraph, p. 295, to read: "Use vacuum during distillation and concentration of the petroleum benzin extract and drying of the residue. In place of either a distillation flask or Soxhlet extraction flask, a large sized aluminum moisture dish may be used." Delete the next sentence, p. 295, beginning with words "In this case"—and ending with words "in a vacuum oven." Under *Alpha Resins (a) Preliminary titration*, p. 295, in par. 1, line 3 insert after word "capacity" the words "by warming." Under (b) *Precipitation of alpha resin*, in par. 1, line 3, after words "side-arm distillation flask" insert the words "or other suitable flask." In line 4, insert after "60°C." the words "using vacuum and." Delete the words "CO<sub>2</sub> and." In line 6, same paragraph, insert words "by warming" after the word "flask." Change the last sentence in par. 2, p. 296, to read as follows: "This quantity is usually sufficient to precipitate the alpha resin from hops having average composition of 16-19% of soft resins." Change the first sentence of last paragraph, p. 296, to read as follows: "In determining the alpha resin content of hops with an exceptionally high or exceptionally low content of soft resins, 1 or 2 ml. more or less of the lead acetate reagent will be required for satisfactory results."

These changes in the method are all made in accordance with suggestions of collaborators in order to simplify and shorten the method without sacrificing accuracy. It was stated by some collaborators that in the determination of the soft and alpha resin content of hops the use of carbon dioxide in the distillation and concentration of the petroleum benzin extracts to prevent oxidation of the resins was time consuming and that the same results could be obtained much more expeditiously by the use of vacuum for concentration. For this reason it is recommended that the use of carbon dioxide be discontinued. A suggested modification of the hard resin determination, which was recommended as a time-saver, was found by most of the collaborators to have no particular merit and hence the procedure in the determination of this resin was not changed.

After the above changes have been made, it is recommended\* that the revised methods, which represent four years of collaborative work and are generally accepted as being satisfactory and efficient, be adopted by the American Society of Brewing Chemists and included in the "Book of Methods" published by that society.

The revised methods are also recommended for adoption as tentative methods for the analysis of hops by the Association of Official Agricultural Chemists.

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 27, 64, 83 (1944).



## REPORT ON BREWING SUGARS AND SIRUPS

By STEPHEN LAUFER (Schwarz Laboratories, Inc., New York City),  
*Associate Referee*

In 1942, George T. Peckham, Jr., then chairman of a subcommittee on brewing sugars and sirups of the American Society of Brewing Chemists, presented a report dealing with the composition and methods of analysis of corn sirups.<sup>1</sup> The report pointed out that corn sirups usually contain reducing sugars which include dextrose and maltose, both fermentable, and higher reducing polysaccharides which may not be fermentable. As analytical procedures Peckham recommended determinations of dextrose equivalent (reducing sugars calculated as dextrose) and of actual dextrose by a modified method of Sichert and Bleyer.<sup>2</sup>

This year, Philip P. Gray, the present chairman of a subcommittee on sugars and dextrans, reviewed the problems encountered in determinations of sugars and dextrans found in brewing sugars and sirups, and in worts and beers, in a report presented at the meeting of the Technical Committee of the A.S.B.C. held in May. The report stressed the importance attached by brewers to the extract and fermentable extract contents of sugars and sirups. For determination of fermentable extract the A.S.B.C. developed a procedure ("Methods of Analysis of the A.S.B.C.," 1940, Brewing Sugars and Sirups) which has been found to give results in good agreement when used by operators who had familiarized themselves thoroughly with the technic in question.<sup>3</sup>

The methods of analysis of the A.S.B.C. for brewing sugars and sirups, including moisture, extract, fermentable extract, and protein, which have been used for many years by brewing chemists with good success,<sup>4</sup> are recommended for adoption by the A.O.A.C. as tentative methods for the analysis of brewing sugars and sirups.

For the determination of sugars in such products, standard methods for sugar analysis can be used. The results can be expressed as equivalent of dextrose, maltose, invert sugar, or sucrose, depending on the nature of the product under test, whether a corn sugar or a malt, invert or sucrose sirup. The methods are available in *Methods of Analysis*, A.O.A.C., 1940, and proper cross references for this purpose will be worked out.

The A.S.B.C. subcommittee intends to conduct collaborative work on the fermentation methods of Stark and Somogyi, which are applicable to the determination of both dextrose and maltose.<sup>4</sup> The results will then be compared with those obtained by chemical methods.

Work is also planned on methods available for determination of dextrin in brewing sugars and sirups.

<sup>1</sup> A.S.B.C. Proceedings, 1942, 106.

<sup>2</sup> Z. Anal. Chem., 107, 328 (1936).

<sup>3</sup> F. P. Siebel, Jr., "Report on Malt Adjuncts," *This Journal*, 23, 174 (1940).

<sup>4</sup> J. Biol. Chem., 142, 519 (1942).

REPORT ON  $pH$  AND ACIDITY OF BEER

By KURT BECKER (J. E. Siebel Sons' Company, Chicago, Ill.),  
*Associate Referee*

During the period from March 1942 to May 1943, work was carried on by a subcommittee of the American Society of Brewing Chemists with a view toward arriving at suitable methods for the determination of  $pH$  and total acidity in beer and related products and materials. Some of the factors that might affect these determinations were investigated individually. Tentative procedures were then selected, and samples of bottled pasteurized beer, ale, and wort were distributed to a relatively small group for collaborative analyses.

In transmitting the results of the collaborative work the Associate Referee again wishes to thank the following individuals and laboratories for their cooperation:

Fred C. Baselt, American Can Co., Maywood, Ill.  
Erik Krabbe, Pittsburgh Brewing Co.  
B. H. Nissen, Anheuser-Busch, Inc., St. Louis  
Frank O. Rickers, The F. M. Schaefer Brewing Co., Brooklyn  
Herman K. Rosenbusch, Stroh Brewery Co., Detroit  
Edward L. Roth, The Geo. Wiedemann Brewing Co., Inc., Newport, Ky.  
Schwarz Laboratories, New York  
J. E. Siebel Sons' Company, Chicago  
Wahl-Henius Institute, Chicago  
Wallerstein Laboratories, New York

 $pH$ , ELECTROMETRIC

Each collaborator was furnished some crystalline, C.P. grade acid potassium phthalate and instructions for preparing a fresh 0.05 M solution to serve for the standardization of  $pH$  meters at  $pH$  4.00 and 20–22°C. Standardization against a uniform buffer has also been commented upon by Rosenblatt (*This Journal*, 26, 301).

Instructions concerning potentiometric technic similar to those given by Bonnar (*Ibid.*, 25, 973) were also issued, and restandardization of  $pH$  meters after any given set of determinations was requested. Above all, collaborators were asked to follow precisely the instructions prepared by the various manufacturers for the proper use of their instruments.

In order to find out what degree of precision and correlation could be attained under these precautions in the measuring of  $pH$  values of relatively simple liquids, readings were first taken on two samples of commercially available, ready-to-use, liquid buffers distributed by the Associate Referee as "unknowns." The corresponding results are presented in Table 1. The agreement must be considered as very good, and the maximum standard deviation of 0.06 as satisfactory.

After these preliminaries,  $pH$  determinations were made on the samples

TABLE 1.—*pH* electrometric checks against "unknown" buffers

Nominal <i>pH</i>	"HARLSCO" NO. 5	"HARLSCO" NO. 9
	4.64	7.38
Collaborator*		
1	4.65	7.41
2	4.60	7.30
3	4.69	7.40
4	4.63	—
6	4.65	7.40
Average	4.64	7.37
Stand. Dev.	0.03	0.06
Max. minus Min.	0.09	0.11

\* Collaborators 1, 2, and 4 used Beckman Model G. Collaborator 3 used Coleman Model 3-C. Collaborator 5 used Coleman Model 3-D. Collaborator 6 used a Leeds & Northrup instrument. All electrode systems: Glass-calomel. All instruments standardised at *pH* 4.00 against 0.05 *M* potassium acid phthalate.

of beer and ale as follows: (a) undiluted, after a more or less complete removal of carbon dioxide through pouring-over or shaking for 60 minutes at room temperature followed by filtration; and (b) diluted with water in the ratio of about 1 plus 9, complete decarbonation being aimed at by following treatment (a) with pipetting of the necessary aliquot into boiling water, boiling for one minute, stirring, and cooling.

With the exception that decarbonation was, of course, unnecessary, similar preparatory treatments were accorded the sample of wort.

The results obtained are shown in Table 2.

TABLE 2.—*pH* of beer, ale, and wort\*

Preparation of sample†	BEER		ALE		WORT	
	(a)	(b)	(a)	(b)	(c)	(d)
Dilution: Sample (%)	100	10	100	10	100	10
Water (%)	0	90	0	90	0	90
Collaborator						
1	4.33	4.45	4.66	4.82	5.12	5.43
2	4.40	4.50	4.70	4.80	5.00	5.30
3	4.38	4.53	4.70	4.86	5.22	5.57
4	4.46	4.42	4.75	4.58	5.12	5.02‡
5	4.21	4.44	4.52	4.72	—	—
6	4.33	4.46	4.60	4.73	5.18	5.42
Average	4.35	4.47	4.66	4.75	5.13	5.43
Stand. Dev.	0.08	0.04	0.08	0.09	0.08	0.11
Max. minus Min.	0.25	0.11	0.23	0.24	0.22	0.27

\* See footnote under Table 1 for instruments used.

† Preparation of sample:

(a) Decarbonation (partial) by shaking or pouring for 60 min. at room temp., followed by filtration.

(b) Step (a) followed by pipetting into boiling water, boiling for 1 minute, stirring, and cooling.

(c) Filtration only.

(d) Filtration followed by procedure (b).

NOTE: The relatively high *pH* values shown by the sample of ale available for collaboration are not typical for this type of product. Generally, ales have *pH* values one to several tenths of a unit below the corresponding values for beers.

‡ Omitted from computations.

All things considered, the agreement can be judged to be fairly satisfactory. Increased numerical *pH* values as the result of dilution are shown. For observations and comments on this effect see also Rohde, (*This Journal*, 25, 270), Bollinger (*Ibid.*, 412), and others.

In evaluating the data in Table 2 and subsequent tables, allowance must be made for the fact that the number of determinations in any given series is insufficient to justify statistical treatment of the results. With this reservation, "average" and "standard deviation" values have, nevertheless, been computed as a matter of convenience.

### *pH*, COLORIMETRIC

While no claims for precision can be made for the colorimetric procedure of determining *pH*, it nevertheless offers certain advantages as a non-standard method, the simplicity and reliability (provided some elementary precautions are observed) of which appeal to the analyst inexperienced in potentiometric technic or lacking that type of apparatus. Such considerations led to the request that colorimetric *pH* determina-

TABLE 3.—*pH* colorimetric checks against "unknown" buffers\*

Nominal <i>pH</i>	"HARLECO" NO. 5	"HARLECO" NO. 9
	4.64	7.38
Collaborator		
2	4.5	7.3†
3	4.5	7.4†
6	4.7	7.5†
Average	4.57	7.40

\* An S.D.C. colorimeter was used by Collaborators 2 and 3. A Taylor colorimeter was used by Collaborator 6.

† Results obtained with phenol red as the indicator. For all other results, the indicator used was bromocresol green.

TABLE 4.—*pH* of beer, ale, and wort\*

Preparation of Sample (For explanation see Table 2)	BEER		ALE		WORT	
	(a)	(b)	(a)	(b)	(c)	(d)
Dilution: Sample (%)	100	10	100	10	100	10
Water (%)	0	90	0	90	0	90
Collaborator						
2	4.2	4.4	4.6	4.7	4.9	5.2
3	4.3	4.5	4.7	4.8	5.2	5.6†
6	4.3	4.4	4.65	4.65	5.2	5.3
Average	4.27	4.43	4.65	4.72	5.10	5.37
Average, electrometric (From Table 2)	4.35	4.47	4.66	4.75	5.13	5.43

\* See footnote under Table 3 for instruments used.

† Results obtained with chlorophenol red. For all other results the indicator used was bromocresol green.

tions be included in the collaborative work. Unfortunately, only three collaborators responded. Their results for the "unknown" buffers as well as the samples of beer, ale, and wort are given in Tables 3 and 4.

On the basis of the very limited number of determinations, the results appear to be in surprisingly good agreement, among themselves as well as when compared with the corresponding values obtained electrometrically. Considering the possibilities for error in the latter procedure, the Associate Referee is inclined to the belief that the inadequacies of the colorimetric procedure are sometimes exaggerated. Reasonably fresh color standards, proper selection of indicators, absence of color interference, and cleanliness of utensils are, of course, essential.

#### TOTAL ACIDITY BY INDICATOR TITRATION

Because of previous experiences indicative of the difficulties in the way of obtaining concordant results when determining the acidity of beer with an inside indicator in the usual manner, collaborators were particularly cautioned to adhere uniformly to the specific instructions issued governing the preparation and dilution of the samples (see under "pH, Electrometric," above) and the details of titration. The use of 0.5 per cent phenolphthalein at the rate of 0.2 ml. per 100 ml. of "10%" diluted sample was prescribed. The first change in color toward a faint pink, judged by comparison with a reference sample identically handled except for the omission of the indicator, was designated as the end point. At the completion of the titration, the pH corresponding to this end point was determined electrometrically.

While the practice of reporting acidity in beer as "% lactic acid," has become firmly established in American brewing, the results of the collaborative work were expressed in terms of the ratio "standard alkali consumed per size of sample titrated." This follows European usage and takes cognizance of the fact that, in all probability, lactic acid plays only a very minor role in beer acidity.

The values obtained for total acidity and pH at the phenolphthalein end points are grouped in Table 5.

About the only satisfaction that can be found in these results is the close agreement (perhaps accidental) in the *average* pH values for the end points in all three sets of titrations, namely 8.21, 8.19, and 8.23. However, this agreement is largely nullified by the divergence among individual results, which show standard deviations of 0.16–0.20, and maximum minus minimum spreads of 0.39–0.55, again illustrating the difficulties, in spite of all precautions taken, inherent in judging phenolphthalein end points when titrating liquids such as beer.

The lack of agreement is even more evident in the acidity values, which show standard deviations of 0.26–0.31 ml. of 0.1 *N* sodium hydroxide per 25 ml. sample, and spreads of 0.65–0.90 between maximum and minimum

results. Such discrepancies, amounting to as much as 25 per cent, are unacceptable to the industry, which expects results to be reproducible with an error not exceeding 5-7 per cent for this determination.

A study of Table 5 shows furthermore that the lack of agreement cannot be attributed wholly to individual differences in judging the end point, since high acidity is not always reported where the pH of the end point is correspondingly high. For example, both Collaborators 1 and 2 titrated the ale to pH 8.30, but found acidities of 3.53 and 4.00, respec-

TABLE 5.—*Colorimetric titration of total acidity in beer, ale, and wort, and pH at phenolphthalein end point*

Preparation of Sample (For explanation see Table 2)	BEER (b)		ALE (b)		WORT (d)	
Dilution: Sample (%)	10		10		10	
Water (%)	90		90		90	
Collaborator	ACIDITY*	FINAL pH	ACIDITY*	FINAL pH	ACIDITY*	FINAL pH
1	4.15	8.25	3.53	8.30	3.88	8.25
2	4.80	8.40	4.00	8.30	4.30	8.30
3	4.28	8.24	3.20	8.07	4.00	8.29
4	4.31	7.97	3.54	8.00	4.33	7.87
5	4.20	7.98	3.40	8.06	3.60	—
6	4.70	8.45	4.08	8.39	3.84	8.42
7	4.70	—	4.10	—	3.70	—
Average	4.45	8.21	3.69	8.19	3.95	8.23
Stand. dev.	0.26	0.20	0.36	0.16	0.31	0.20
Max. minus Min.	0.65	0.48	0.90	0.39	0.73	0.55

\* Acidity expressed as ml. of 0.1 *N* NaOH/25 ml. of beer, ale, or wort, using 0.5% phenolphthalein indicator at the rate of 0.2 ml./100 ml. of diluted sample, and titrating to appearance of first pink color.

tively; and, in the case of the sample of beer, Collaborator 4 titrated to pH 7.97, but found a higher acidity, namely 4.31, than Collaborator 1, who titrated to pH 8.25 for an acidity of only 4.15.

In this connection, mention can be made of the fact that various collaborators and investigators have, at different times, reported pH values for the phenolphthalein end point in beer titration varying from 7.9 to 9.1, depending upon conditions, particularly the concentration of indicator and degree of dilution, if any, of the sample. For 10 per cent dilution, and using 0.5 per cent phenolphthalein at the rate of 0.2 ml. per 100 ml. of diluted sample, the preferred range seems to lie between pH 8.2 and 8.6, inviting, of course, closer definition.

Confronted with unsatisfactory agreement, some of the collaborators made numerous determinations. These revealed another disturbing element, namely erratic results from different bottles of the same sample,

suggesting that perhaps acidity and pH might be affected by traces of alkali progressively introduced from the walls of bottles subjected to repeated washing with caustic soaker solutions.

Further work is required to clarify the situation.

#### ACIDITY BY POTENTIOMETRIC TITRATION

One would expect that the potentiometric technic would avoid the drawbacks of colorimetric determination of acidity. However, the study of data furnished by the six collaborators who found it possible to conduct potentiometric titrations makes it very clear that attention to detail

TABLE 6.—*Points of inflection on potentiometric acidity curves*

SAMPLE	CONCENTRATION	APPARENT POINTS OF INFLECTION*		COMMENTS ON SHAPE OF DIFFERENTIAL CURVES
		COLLAB.	AT pH—	
	<i>per cent</i>			
Beer	10	A	8.25	fairly definite
	10	B	7.82, 8.30	fair, but ambiguous
	10	D	8.18, 8.48, 8.77	unsatisfactory
	50	C	8.3	very definite
	100	B	7.02, 7.33	unsatisfactory
	5	E	6.40, 8.25	erratic
Ale	10	B	7.82	definite
	10	D	8.13	fair, but very flat
	100	B	7.72 to 7.94	very flat, hardly satisfactory
	50	C	8.2	very definite
Wort	10	A	8.10, 8.43, 8.89	erratic, ambiguous
	10	B	7.93, 8.46	erratic, hardly satisfactory
	50	C	8.4	quite definite
	100	B	7.85	fair, but very flat
	10	D	8.13	ambiguous, erratic

\* Ascertained from the original titration data, and representing pH values where the ratio  $\frac{\Delta \text{pH}}{\Delta \text{alkali added}}$  was a maximum.

NOTE: Collaborator E titrated with 0.01 N, all others with 0.1 N alkali.

and familiarity with technic are prerequisites for realizing such expectations.

In potentiometric work, one has the choice of two ways of defining the "end point": by accepting the titer corresponding to the point of inflection, most conveniently found by plotting the differential curve (showing ratios of "increment in pH" to "increment of alkali added"), or by titrating to a selected, fixed pH value.

From the viewpoint of the first of these two criteria, Table 6 presents excerpts from the potentiometric data, showing the points of inflection determined by calculation from the original titration data of the various collaborators. These values cover a range from pH 6.40 to 8.89. Obviously, the readings from some of the titrations were such as to make the determination of points of inflection practically impossible. The generally un-

satisfactory nature of this compilation, notwithstanding, there is encouragement in the fact that one collaborator, known to be particularly proficient in details of potentiometric technic, was able to obtain non-ambiguous, definite differential curves, and from them points of inflection within the relatively narrow range of 8.2-8.4.

It is to be noted that all but one collaborator titrated with 0.1 *N* solutions. Greater accuracy from the use of 0.01 *N* solutions has been pointed out by Rohde (*loc. cit.*) and by Nissen and Thompson.<sup>1</sup>

The data were also interpreted from the second criterium by interpolating the titers to a fixed *pH*, chosen more or less arbitrarily as 8.2. (Table 7).

TABLE 7.—Acidity titrated potentiometrically to *pH* 8.2

SAMPLE	CONCENTRATION	COLLABORATOR	ACIDITY, ML. 0.1 ALKALI/ 25 ML. SAMPLE, TO <i>pH</i> 8.2*
	<i>per cent</i>		
Beer	10	A	4.08
	10	B	4.40
	10	D	4.50
	100	B	4.61
Ale	10	B	4.06
	10	D	4.13
	100	B	4.38
Wort	10	A	3.68
	10	B	4.61
	10	D	3.48
	100	B	4.58

\* From the original titration data, interpolated where necessary.

NOTE: The much higher acidity values of wort obtained by Collaborator B must be considered in the light of possible spoilage, in spite of the fact that the samples were double-pasteurized.

Again, there is room for improvement as regards reproducibility of results, even if the sample of wort analyzed by Collaborator B is omitted from consideration.

In the course of the collaborative work, some studies were also made of methods for decarbonating beer in preparation for *pH* and acidity determinations by vacuum and aeration. Effects of varying the boiling time, concentration of sample, and concentration of indicator were also observed. These are commented upon in the 1942 and 1943 (mimeographed) Reports of the A.S.B.C. Subcommittee on Acidity and *pH*.

#### GENERAL OBSERVATIONS

As regards *pH*, its inclusion in the analysis of beer and wort is of significance with respect to whether or not proper hydrogen-ion concentration for enzyme action prevailed in the mash; it reflects certain characteristics of the yeast used in wort fermentation, gives warning of possible



deterioration of the finished product, and allows some predictions as to expected biological and protein stability. Broadly speaking, pH values also distinguish between certain types of beer, ale, etc.

From experience gathered to date, there appears to be no serious detriment to establishing suitable, reliable methods for determination of pH in beer, etc., but some details of procedure remain to be standardized. Both electrometric and colorimetric procedures merit consideration in this connection.

The inclusion of acidity values in the analyses of beer and wort is traditional, and indicated for reasons similar to those mentioned above in relation to pH. Incidentally, it is well to bear in mind that, because of differences in chemical composition, different beers often fail to show correlation between acidity and pH values, i.e., relatively low pH may accompany high acidity and vice versa.

The complex nature of beer acidity has been pointed out by numerous investigators. Recently Nissen and Thompson confirmed the concept that phosphates play a predominant role in beer acidity by comparing potentiometric curves of beer with those of solutions of maltose (1.5 per cent), dextrin (2.2 per cent), lactic acid (0.15 per cent), and phosphoric acid (0.15 per cent). At the risk of slight exaggeration, one might say that the term "acidity" with respect to beer is a misnomer, since the operations surrounding its determination really constitute a measuring of the buffering capacity.

The Associate Referee is not ready at this time to make recommendations regarding methods for determining acidity and pH in beer.

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## REPORT ON SULFUR DIOXIDE IN BEER

By L. V. TAYLOR (Research Department, American Can Company,  
Maywood, Ill.), *Associate Referee*

A previous paper by the Associate Referee (*This Journal*, 23, 189) showed that the Monier-Williams method, with slight modifications, yielded reliable and reproducible results for the determination of sulfur dioxide in malt beverages. The results of this collaborative work and the results of an earlier investigation of sulfur dioxide methods for beer and wine (*Ibid.*, 20, 610) led to the Associate Referee's recommendation that the modified Monier-Williams method be adopted by the Association for the determination of sulfur dioxide in malt beverages. This recommendation was accepted, and the method now appears as official (first action) in *Methods of Analysis*, A.O.A.C., 1940, 154.

Prior to final acceptance it was considered advisable to subject the procedure to further study. This report, therefore, deals with an investigation and comparison of other methods that have appeared in the recent litera-

ture with the A.O.A.C. procedure. The actual investigational work was carried out by E. D. Sallee in the Food Analysis Laboratory of the American Can Company Research Department. In addition to the A.O.A.C. procedure the methods studied were: (a) direct titration methods adapted from work by Bennet and Donovan<sup>1</sup> on citrus juices and suggestions taken from an article by Mapson<sup>2</sup> describing his work on ascorbic acid assays in the presence of sulfur dioxide; (b) a steam distillation procedure adapted from a method employed by Kirkpatrick<sup>3</sup> in his work on ascorbic acid and sulfur dioxide in fruit juices; and (c) the Nissen-Petersen<sup>4</sup> modification of the Monier-Williams method.

*Direct Titration Methods.*—Attempts were made to devise a direct iodine titration procedure that would be applicable for sulfur dioxide measurement in beer. Such a procedure would necessarily entail the use of at least two titrations: First, the titration of total reducing substances including sulfur dioxide; and, second, the titration of the natural reducing substances after the sulfur dioxide has been eliminated by evolution or by formation of some compound that does not react with iodine. The difference between the two titrations represents the sulfur dioxide measurement. In the Associate Referee's laboratory several variations of technic were tried, none of which was successful. It was endeavored to measure the total iodine reducing power in samples of beer acidified with hydrochloric or sulfuric acid and in beer treated with an excess of sodium hydroxide and, after standing for various lengths of time up to 30 minutes in a sealed vessel, subsequently acidified and titrated immediately. Likewise, attempts were made to titrate the natural reducing substances alone after evolution of the sulfur dioxide by acidifying followed by agitating or boiling and sweeping with carbon dioxide. Attempts were also made to estimate the natural reducing substances in the beer after the sample had been treated with sodium hydroxide, acetone, and then an amount of sulfuric acid equivalent to the alkali employed. The concentration of acetone, as well as the pH of the mixture after sulfuric acid addition, was varied over a considerable range in an effort to strike a suitable combination for the optimum formation of the sulfur dioxide-acetone compound. In all cases the titrations were unsatisfactory because no definite reproducible starch-iodine end point could be obtained.

The sulfur dioxide content normally found in present commercial beers is so low that a large sample must be used in order to deal with sufficient sulfur dioxide to titrate. In the six brands of beer tested the iodine used by the reducing substances was at least ten times greater than the iodine needed for titration of the sulfur dioxide alone. With such a high "blank"

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<sup>1</sup> *Analyst*, 68, 140 (1943).

<sup>2</sup> *Biochem. J.*, 36, 196 (1942).

<sup>3</sup> *J. Soc. Chem. Indus. Trans.*, 60, 226 (1941).

<sup>4</sup> *Ind. Eng. Chem., Anal. Ed.*, 15, 129 (1943).

titration the end point would have to be very sharp and clearly defined in order to obtain any reasonable degree of accuracy. Experience in this laboratory and consideration of the nature of present commercial beers indicate that direct iodine titration procedures are not feasible for sulfur dioxide determinations.

*Steam Distillation.*—The steam distillation technic tried was practically the same as that used by Kirkpatrick<sup>3</sup> for the determination of sulfur dioxide in citrus juices. The apparatus used differed only in the size of the Kjeldahl flask used for the sample container. In Kirkpatrick's work a 10–25 gram sample contained in a 100 ml. Kjeldahl flask was steamed and swept with nitrogen for a total of 7–8 minutes, which absorbed the sulfur dioxide in three per cent hydrogen peroxide. Owing to the low concentration of sulfur dioxide in the beer tested in this work it was necessary to use a 300 ml. sample acidified with 20 ml. of hydrochloric acid contained in a 750 ml. Kjeldahl flask. The distillation and sweeping were carried on for as long as 90 minutes or until the condensed steam filled the sample flask. In all instances the results were variable and were less than 25 per cent of the values found by the A.O.A.C. procedure.

*Nissen-Petersen Modification of Monier-Williams Method.*—This modification involves essentially the manner of neutralizing the peroxide solution and subsequent titration of the absorbed sulfur dioxide. In the Nissen-Petersen technic the peroxide is neutralized to a pH of 4.0 by potentiometric titration with sodium hydroxide solution and, after absorption of the sulfur dioxide, again titrated to a pH of 4.0 electrometrically with 0.01 *N* sodium hydroxide. The peroxide may be prepared rapidly and is ready for immediate use.

In the A.O.A.C. procedure the peroxide is prepared by neutralization to the bromophenol blue end point with barium hydroxide solution and the sulfate originally present is removed by precipitation and filtration. The presence of excess barium ion in the peroxide after this treatment leads to a negative error in the volumetric estimation of approximately 0.3 mg. of sulfur dioxide.

Six samples of beer representing six different brands were obtained locally, and sulfur dioxide determinations were made both volumetrically and gravimetrically by the two procedures. The values obtained are shown in the table. Analyses were made simultaneously in order to be sure that the samples were identical at the time of analysis. Each value is the average of duplicate determinations and has been corrected for the blank determination. The data presented show that the two methods agree very well. As Nissen and Petersen pointed out, when the peroxide is neutralized with barium hydroxide, due to excess barium ion, the volumetric results are in general slightly lower than the gravimetric checks. In the experience of the Associate Referee, however, the error is no greater than the variations between duplicate samples. In the more reliable gravimetric

technic the estimation of sulfur dioxide is not affected by the modification except that the sulfuric acid originally present in the peroxide is not removed, thus causing a variable and higher blank than when the A.O.A.C. procedure is used. Blank determinations were made in each method for every new lot of peroxide employed. The gravimetric blank determinations for the peroxide neutralized by the Nissen-Petersen procedure were equivalent to 1.1–4.2 mg. of sulfur dioxide per liter of beer; while those for the A.O.A.C. technic were constant and equivalent to 0.7 mg. of sulfur dioxide per liter. Although the modification has a possible advantage of time saving and greater precision in the volumetric estimation of sulfur dioxide, the accuracy of the more reliable gravimetric check is not improved and in many cases it may be endangered.

The accuracy and reproducibility of the present A.O.A.C. method for the determination of sulfur dioxide in beer have been demonstrated. On the basis of past experience, investigations of other available methods for the determination, and the difficulty in preparation and delivery of samples of a known sulfur dioxide content, the Associate Referee considers that further collaborative study is unwarranted. It is recommended, therefore, that the method as it appears in *Methods of Analysis, A.O.A.C.*, 1940, 154, be made official (final action) and that work on the problem be discontinued.

*Comparison of Nissen-Petersen method with A.O.A.C. procedure for determination of  $\text{SO}_2$  in beer (mg.  $\text{SO}_2/\text{l.}$ )*

BRANDS OF COMMERCIAL BEER	NISSEN-PETERSEN MODIFICATION		A.O.A.C. PROCEDURE	
	VOLUMETRIC	GRAVIMETRIC	VOLUMETRIC	GRAVIMETRIC
A	8.2	6.3	7.6	7.2
B	5.3	5.2	4.9	5.6
C	16.2	13.5	13.2	13.5
D	3.4	3.6	3.1	3.5
E	25.8	24.4	23.3	24.5
F	0.7	0.2	0.0	0.5
A + 57.7 mg. $\text{SO}_2/\text{l.}$	65.3	66.4	63.5	65.4

## REPORT ON COLOR AND TURBIDITY IN BEER AND WORT

By B. H. NISSEN (Brewery Division, Anheuser-Busch, Inc.,  
St. Louis, Mo.), *Associate Referee*

During the past several years, the Associate Referee has acted as chairman for both the color and the turbidity committees for the American Society of Brewing Chemists. In this work he has tested not only the customary Lovibond method for color, but also such other methods as were found to be recommended for the color determination of beer.

In addition, during 1942, extensive study was made on the use of photoelectric instruments for the measurement of color, which resulted

in a paper entitled "Color and Turbidity in Beer and Wort."<sup>1</sup> These photoelectric measurements revealed that in most color work it is necessary to consider turbidity unless the solution examined is brilliantly clear. Accordingly, an attempt was made to work out a photoelectric method whereby both color and turbidity could be determined at the same time. By the use of certain types of instruments, such as the colorimeter used in these experiments, the Cenco-Sheard-Sanford photelometer, it was observed that reproducible curves could be prepared from which the color and turbidity could be obtained. This work, with summary, is given in detail in the paper.<sup>1</sup>

At present, the Lovibond Tintometer, with Lovibond glasses, series No. 52, and a  $\frac{1}{2}$ " cell is the official A.S.B.C. method for color in beer. After extensive investigation by the committee on this well-known and widely used type of tintometer, it was found that the slides of this instrument vary a great deal. In many instances discrepancies were found between glasses marked to read the same. For example, in the following two sets of Lovibond glasses, series No. 52, compared with the most recently obtained set of standards, wide discrepancies appear, as indicated in the table below.

*Duplicate Lovibond Glasses*

STANDARD MARKED TO READ—	ACTUAL READING, 1ST SET	ACTUAL READING, 2ND SET
1.5	1	2.1
2	$\left\{ \begin{array}{l} 2 \\ 2 \end{array} \right.$	$\left\{ \begin{array}{l} 0.9 \\ 1.8 \end{array} \right.$
3	3.25	2.9
5	5.25	4.5
6	5.50	5.6
7	6.50	6.5
8	7.50	7.5
9	9.0	8.5
16	16	15

Not only were these slides inaccurate, as indicated in the table, but the duplicate slides were found not to agree with each other, especially in the lower values, which is the range most often used for the color of beer.

It was also noticed, for example, that several of the fractional slides were so inaccurate as to make their use very questionable. In another instance, one slide of a particular value appeared brighter in color than another of a slightly lower value. Furthermore, any practice of superimposing fractional slides over each other to obtain desired readings tended to give a grayer cast in color, due no doubt to the reduced transmission of light. These and other variations appeared to exist both in the newer slide type Lovibond tintometer, as well as in the older models even with

<sup>1</sup> *Proc. Am. Soc. Brew. Chemists*, May 25-27, 1942.

the most generally accepted light sources, many of which were examined. Since no better method seems to be available, however, the Lovibond tintometer continues to be the accepted and official method for color work in the brewing and malting industry.

Other methods suggested for color consist of matching solutions such as iodine or special dyes. The iodine solution used consists of 0.01 *N* iodine diluted in water in a series from 1 to 50 ml. Photoelectric examination of these iodine solutions indicates that concentrations above 6 ml. per 100 ml., equivalent to approximately 4.25° Lovibond, match the beer quite closely, but that in concentrations below the 6 ml. per 100 ml., the color varies increasingly from that of beer, becoming greener in shade. For greater permanence iodine color standards must be kept hermetically sealed and away from the light. Even then, they tend to change.

Several types of dye solutions were tested out, namely, the Brand dye, the American Public Health Color Standards, and the Cobalt-Iron-Copper Pharmacy Colors. Difficulties resulted, however, in the selection of suitable colors and proper matching for all types of beer and wort. Effort, of course, was directed towards finding a suitable mixture covering most of the beers and worts usually encountered. Of all the color standards prepared in this manner, those employing the inorganic salts seemed to offer the most hope. It appeared that reproducible sets of such standards could be prepared once the initial intensity series had been worked out. However, it was observed that a progressive intensity series could not be used. Each standard of the series had to be prepared separately, and consideration given not only to the overall intensity change, but also to changes in the relative proportions of one color to another in the mixture. This naturally involves an enormous amount of detail preparation.

Recently efforts have been directed towards preparing fairly permanent standards by using the Brand dyes prepared in accordance with the method suggested in the following turbidity discussion. These standards are more permanent than the iodine standards and more easily prepared.

The aim, however, should be to develop some type of photoelectric colorimeter that might serve to register accurately the color of beer and wort, and thus eliminate the ever present uncertainties experienced with the human element, as well as the discrepancies referred to previously from use of present color methods.

It was soon observed that it would also be desirable to give consideration to turbidity. No standard method for turbidity has yet been accepted as official by the A.S.B.C., but the committee has worked out and prepared a recommended method independent of the photoelectric procedure referred to earlier. Studies of the keeping qualities of beers and other malted beverages under varying conditions of storage and temperature are valuable. If a standard procedure for measuring turbidity like the following were used, instead of the indefinite designation of "haziness" or

"cloudiness," it would be possible to have numerical data from many sources available for comparative purposes.

Preliminary work was confined to the study of the relative merits of methods now in use and to a determination of the value and reproducibility of turbidity measurements by the most generally used methods, which usually employ suspensions of fullers' earth and water. A method for turbidity measurement, as well as standards of low turbidity, was prepared. This method is somewhat similar to the method used for water analysis by the American Public Health Association, and consists of dispersions of fullers' earth in water arranged in series from 0 to 50 p.p.m. The following is a description of the method now recommended.

#### DETERMINATION OF TURBIDITY

Match the haziness of the beer sample in a wide-mouthed bottle against a set of standard bottles made up to definite turbidities such as the following, 0, 1, 2, 4, 6, 8, 10, 12, 15, 20, 30, 40, 50, and 100 p.p.m. turbidity.

Make the comparison by holding the standard bottle and the sample at arm's length, sighting toward a good light, north daylight preferred, and focusing on a black horizontal band such as a metal frame of a window. Shake the standard sample just before matching. Immerse the sample and standard in water to which a drop or two of a suitable wetting agent has been added, which effectively removes the sweating that occurs if the sample is cold. (The matching of the clouding effect against the dark band is the result desired. After a little practice, various operators read in close agreement. Values between the standards can be interpolated.)

This method is advantageous in that turbidities at any temperature, either cold or warm, can be taken quickly, before the turbidity changes. The 16 oz. bottle allows a complete 12 oz. sample to be taken at one time, and permits shaking and mixing, as a sediment is often present at the bottom.

The bottles used are regular 16 fluidounce, wide-mouthed, square type, tall, clear, glass sample bottles,  $2\frac{1}{2}'' \times 2\frac{1}{2}'' \times 5\frac{1}{2}''$  to the shoulder. The liquid is colored with a mixture of dyes to approximate the average color of beer. Mercuric chloride is added as a preservative. The final turbidity standards are prepared from a standardized 100 p.p.m. mixture. The bottles are well filled and closed with screw caps. New standards should be made up about once a month, as the standards tend gradually to become more turbid and therefore inaccurate, especially at very low turbidities.

The preparation of the 100 p.p.m. stock solution is as follows:

Mix 1 teaspoonful of Pears White Precipitated Fullers' Earth, A & F Pears, Ltd., London, with 1.5 liters of distilled water and stir five times in 24 hours. Carefully pour off the supernatant liquid to eliminate the coarse material that settles out. Then dilute this suspension to 100 p.p.m. turbidity, using the government candle method and equipment (Jackson turbidimeter, etc.). Then make up the standard bottles from this 100 p.p.m. suspension.

To distilled water in a 500 ml. volumetric flask, add the quantity of 100 p.p.m. turbidity suspension required, approximately 5 ml. of filtered Brand dye solution,

and 5 ml. of saturated  $\text{HgCl}_2$ . Make up to mark. Prepare aliquot dilutions for the desired p.p.m. standards.

To simulate the color of beer add the required amount of Brand dye solution. Prepare this solution as follows:

<i>gram</i>	<i>gram</i>
0.05 Patent Blue V. A. ....	0.12 Resorcin Brown G
0.20 Amaranth W N. ....	0.80 Tartrazine C. Extra

(These dyes were secured from General Dyestuff Corp., Chicago, Illinois.)

Dissolve these dyes in 20% ethyl alcohol in a liter flask and then dilute to 1 liter with 20% alcohol. (It may be necessary to vary the concentrations of each dye slightly to match any particular sample.)

This procedure has worked out very well for all the usual turbidity measurements, and it suffices fairly well with certain precautions in preparation and with monthly renewals to compensate for change in values with age.

In the comparison of turbidity values, however, with the photoelectric procedure described<sup>1</sup> some differences have been observed due to type of turbidity. Ordinarily by far the greater number of turbidity comparisons in the case of beer and wort examination fall under the protein type, but some instances of dextrin turbidity resulting from frozen beer, and yeast turbidity due to fermentation are encountered.

When such turbidities are compared with standards prepared with fullers' earth, it is found that the protein turbidities appear to give good agreement, both in the visual as well as by the photoelectric procedure. The dextrin and yeast turbidities, however, seem to give lower readings with the photoelectric method for the higher turbidity values, and higher readings for turbidity values in the neighborhood of 5-10 p.p.m. as compared with the visual procedure. Just what effect this slight variation will have on a method of this kind would have to be determined. Other than this, the method seems to be quite workable, and has proved valuable in many tests.

Additional experimentation has been conducted with samples of KWKSol Bentonite (American Colloid Company) as the turbidity suspension. After preliminary preparation and settling out of the coarser particles, it appears that the standards prepared from this material remain stable for much longer periods than those prepared from fullers' earth. This particular grade of Bentonite has the property of not caking or balling as much in suspension as regular Bentonite of the Volclay type, although the latter may even be of much finer particle size.

As the matter now stands, the accepted method for color is the Lovibond procedure, while for turbidity, the above procedure is being recommended. As mentioned previously, however, an effort is being made to combine these two procedures in a photoelectric method.



## REPORT ON CEREAL FOODS

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

No report was published by the Referee in the 1942 *Journal* of the Association. Three reports were published by associate referees: Collatz, 26, 107, on electrolytic methods for the determination of hydrogen-ion in cereal products; Etheredge, 26, 214, on the determination of starch in cereal products; and Walker, 26, 305, on methods for moisture and fat by acid hydrolysis on fig bars and raisin-filled crackers. The recommendations of the Referee on these three papers are included in this report.

Attention is directed to the recommendation for appointment of an associate referee to conduct studies on the qualitative and quantitative methods for the determination of bromates in bromated flour and bromated whole wheat flour.

E. G. Bayfield has called attention to the possible value of the application of apparent viscosity measurement to the entire range of flour in this country. Since the present official method may need modification for the high protein flours it is recommended that an associate referee be appointed to study this subject.

Most of the associate referees have neither reported nor submitted any comments. Associate Referee Harris has published a number of papers recently on quality study showing relationship between durum wheat and finished macaroni, but has nothing definite to present on the identification of the type of raw materials used in macaroni. Associate Referee Taylor reports some progress on the formulation of methods for ether extract and crude fiber in soybean flour among members of the soybean industry.

A rapid method for the determination of tartrazine in macaroni products (*This Journal*, 26, 95), should be included following the procedure for color in macaroni products, p. 237, *Methods of Analysis*, A.O.A.C., 1940).

The following recommendations are based on the work of the associate referees:

## RECOMMENDATIONS\*

It is recommended—

(1) That the method for the determination of iron on which collaborative work was reported by the referee be adopted as tentative (*This Journal*, 27, 86), and that study be continued and conference held with other referees studying methods for iron.

(2) That the method for the determination of calcium in cereals on which collaborative work was reported by the Referee be adopted as tentative (*Ibid.*, 87), and that study be continued.

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 27, 66, 85 (1944).

(3) That the study of methods for the detection and determination of rye flour in rye bread and mixtures of cereal flour be continued.

(4) That the electrometric procedure for the determination of hydrogen ion concentration in cereal products (*Ibid.*, 26, 109) be adopted as tentative, and that study be continued.

(5) That the Hopkins revision of the Mannich-Lenz procedure for the determination of starch in cereals submitted to collaborators by the associate referee this year be adopted as tentative (*Ibid.*, 27, 87), and that studies on the determination of starch be continued.

(6) That further study be given to the determination of acidity of fat in grain, flour, corn meal, and whole wheat flour and to the correlation of this factor with unsoundness.

(7) That further studies be made of the determination of reducing and nonreducing sugars in flour by the method given in section 20, p. 215 of *Methods of Analysis, A.O.A.C.*, 1940, and of its application to the determination of sugar in bread and other cereal products.

(8) That the study of the tentative method for the determination of benzoyl peroxide in flour (*Ibid.*, 44, 223) be continued.

(9) That studies be continued on methods for the determination of available carbon dioxide in self-rising flour containing added calcium carbonate.

(10) That methods for the determination of lactose in bread (*This Journal*, 25, 630) be further studied.

(11) That the tentative method for the estimation of milk fat in bread (*Methods of Analysis, A.O.A.C.*, 1940, 65, 229) be further studied.

(12) That the methods proposed by the associate referee for the determination of proteolytic activity of flour be further studied.

(13) That a study be made of the applicability of methods in the chapter on wheat flour to the determination of moisture, ash, protein, crude fiber, and ether extract in soybean flour.

(14) That studies be made of the detection and determination of soybean flour in cereal products by immunological methods or other suitable means of estimation.

(15) That studies of the determination of added inorganic materials in phosphated and self-rising flours be continued.

(16) That the methods referred to in *This Journal*, 25, 83-84, for the determination of unsaponifiable matter and sterols in noodles be studied to determine their applicability to other foods containing eggs.

(17) That studies of methods for the determination of albumin nitrogen in noodles and other farinaceous egg-containing products be continued.

(18) That methods for the determination of moisture and fat by acid hydrolysis in fig bars and raisin-filled crackers (*Ibid.*, 26, 305) be adopted as tentative and studies continued.

(19) That further studies be made of the modified distillation method (benzene) for the determination of moisture in all flour-like products containing sodium bicarbonate as one of its constituents (*Ibid.*, 25, 649).

(20) That methods for the determination of bromates in flour be studied.

(21) That the method for apparent viscosity measurement of flour be studied.

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## REPORT ON IRON AND CALCIUM IN CEREALS

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The official and tentative methods of analysis of the A.O.A.C. include no methods for the determination of iron and calcium in cereals. With the promulgation of the standards for enriched flour, enriched bromated flour, enriched self-rising flour, and enriched farina (Fed. Reg. May 27, 1941), as amended except for enriched farina (Fed. Reg. July 3, 1943), the proposed order for enriched bread (Fed. Reg. August 3, 1943) and the regulations for special dietary use (Fed. Reg. November 22, 1941) the necessity for the Association to have methods for iron and calcium in cereal products is apparent.

Recent publications by Fortune and Mellon,<sup>1</sup> Woods and Mellon,<sup>2</sup> and Andrews and Felt<sup>3</sup> cover the references to the literature on this subject sufficiently so that further references seem unnecessary.

### DESTRUCTION OF ORGANIC MATTER AND SOLUTION OF IRON

Various means for destruction of the large proportion of organic matter in these products are known. The following principles seemed most appropriate and were tried in this study: (1) ash by the official method for determination of ash in flour; (2) ash with added sodium hydroxide as a fixative essentially as presented by Hoffman *et al.*,<sup>4</sup> (3) ash with added magnesium nitrate solution mixed with the cereal, and (4) wet ashing. Without discussion of the details of these various procedures and the factors involved in the choice of a procedure, the Associate Referee selected the official method for determination of ash in flour for this collaborative study. In connection with ashing cereal products special consideration was given to loss of iron by volatilization, especially in the presence of added salt, and the incomplete solution of iron due to incomplete destruction of all carbon or the conversion to partially insoluble iron salts. The different types of iron salts, the calcium salts introduced as carriers in

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<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 10, 60 (1938).

<sup>2</sup> *Ibid.*, 13, 551 (1941).

<sup>3</sup> *Cereal Chem.*, 18, 819 (1941).

<sup>4</sup> *Ind. Eng. Chem., Anal. Ed.*, 12, 454 (1940).

the enrichment mixture, and the various characteristics of the cereal products are important factors in the completeness of the removal of the carbon and the solution of the iron. To insure, so far as possible, the elimination of these factors an "ash aid," such as the magnesium nitrate given in the procedure, has been found effective. The iron in the ash is dissolved in concentrated hydrochloric acid by evaporation to dryness on the steam bath, later taken up in a limited amount of concentrated hydrochloric acid, reduced to ferrous iron, and buffered, and a colored solution is developed by orthophenanthroline or alpha-alpha dipyridyl for measurement of the iron content. This study confirms the statement of Woods and Mellon that potassium sulfocyanate is inferior to orthophenanthroline and alpha-alpha dipyridyl. No significant difference was indicated for orthophenanthroline and alpha-alpha dipyridyl so that a choice is given in the procedure; however, the higher absorption at the same concentration for orthophenanthroline and the possibility of less interference are factors in its favor. Any of the numerous accurate instruments, such as spectrophotometer, photoelectric colorimeter, and neutral wedge photometer, may be used for measurement of the concentration of iron in the solution.

#### PRECISION AND ACCURACY

In order to demonstrate the range of precision and accuracy of the method the results in Table 1 are presented. The three products, flour,

TABLE 1.—*Iron in flour, semolina, and whole wheat flour*

WHITE FLOUR	SEMOLINA	WHOLE WHEAT FLOUR
mg./lb.	mg./lb.	mg./lb.
5.6	4.9	16.8
5.9	4.8	16.8
5.8	4.7	16.8
5.8	4.6	17.2
5.8	4.7	16.7
5.8	4.6	16.8
5.5	4.6	16.8
—	4.6	17.0
—	—	16.9
—	—	17.1
—	—	—
Av. 5.7	Av. 4.7	Av. 16.8

semolina, and finely ground whole wheat flour, contain no added iron other than that introduced during milling, which possibly represents a minimum distribution factor. These results were obtained in connection with other work on iron determinations extending over a period of many months. Different reagents and different mufflers were used with some variations in ashing time at 550°C.

Samples of phosphated and self-rising flour containing added iron salt calculated according to the composition of the ingredients to have 6.9 and 9.7 mg./lb., respectively, were analyzed. The results are shown in Table 2.

The average results on phosphated flour (Table 2) indicate a 100 per cent recovery, and those on the self-rising flour 96 per cent recovery.

Samples of flour and an enriching concentrate containing sodium iron pyrophosphate were weighed directly in the crucible used for ashing to

TABLE 2.—*Iron in phosphated and self-rising flour*

PHOSPHATED FLOUR	SELF-RISING FLOUR
mg./lb.	mg./lb.
6.9	9.2
7.1	9.2
6.9	9.3
7.3	9.5
7.5	9.5
7.6	9.5
6.9	9.4
6.8	9.4
6.3	9.1
6.4	—
6.7	—
6.7	—
—	—
Av. 6.9	Av. 9.3

eliminate any distribution factor in mixing. Semolina was treated in similar manner. The recoveries on the plain flour were 100, 93, 90, 105, 98, 96, 94, 90 per cent, average 96 per cent, and on the semolina, 96, 96, 96, 96, 103, 96, 97, 96, 96, 97, 95, 94 per cent, average 96 per cent. Semolina mixed in the crucible used for ashing with 10 ml. of iron solution containing 0.1 mg. iron gave an average of 98 per cent recovery. These results, together with those in Tables 1 and 2, indicate satisfactory precision and accuracy.

#### EFFECT OF SALT ON IRON RECOVERY

Since bread contains salt the effect of added salt on the iron recovery is presented in Table 3. The comparison of the iron content of breads made from the same ingredients with and without salt was found to be unsatisfactory for this purpose; therefore semolina was mixed with salt solution equivalent to 2 per cent and also with dry salt. Flour and enriched flour doughs, with and without salt, of the usual bread formula were weighed into crucibles used for ashing.

The sodium hydroxide fixative in Hoffman's procedure is used to eliminate the possibility of iron loss caused by the presence of salt. A sample of enriched bread was analyzed by the procedure used in this study and

TABLE 3.—*Recovery of iron from semolina and from bread dough and enriched bread dough, all with and without salt*

SEMOLINA		DOUGH		ENRICHED DOUGH	
WITHOUT SALT	WITH SALT	WITHOUT SALT	WITH SALT	WITHOUT SALT	WITH SALT
	mg.	mg.	mg.	mg.	mg.
Av. 8	0.1043	0.1051	0.1027	0.2690	0.2691
determinations,	0.1002	0.1060	0.1002	0.2700	0.2683
0.1031 mg.					
	Av. 0.1028	0.1055	0.1015	0.2695	0.2687
Recovery (%)	99.5		96		99.8
Semolina with dry salt		0.1051	Recovery (%)	102	
		0.1045			
		0.1051			

TABLE 4.—*Iron in enriched bread*

PROCEDURE USED IN COLLABORATIVE STUDY	NaOH FIXATIVE
mg./lb.	mg./lb.
13.3	13.4
13.3	13.2
13.2	13.8
	13.2
Av. 13.3	13.3
	13.0
	Av. 13.3

by a modification of the procedure with a sodium hydroxide fixative during ashing (Table 4).

These results (Tables 3 and 4) indicate little if any effect of the salt on the iron recovery under the condition of these experiments.

#### DESCRIPTION OF SAMPLES

Four samples and an iron solution of known iron content were prepared and submitted for collaborative determination of iron. Sample No. 1 was a phosphated flour and No. 4 a self-rising flour prepared by mixing the ingredients 18 hours in a ball mill with iron-free marbles; No. 2 was a finely ground wheat flour and No. 3 was enriched bread. Samples No. 1 and No. 4 were calculated to have 6.9 and 9.7 mg./lb., respectively. Instructions were issued to follow the method given below.

#### IRON IN FLOUR AND BREAD PREPARATION OF SAMPLE

Slice bread, allow to air-dry until in equilibrium with air, and crush on wooden

surface with wooden rolling pin to approximately 20-mesh size. (Grinding may be done in mill if experiments show no increase in Fe due to grinding. In general, grinding in mills increases the iron content.)

#### REAGENTS

(a) *Orthophenanthroline solution*.—Dissolve 0.1 gram of orthophenanthroline in about 80 ml. of water at 80°, and after cooling dilute to 100 ml.

(b) *Alpha-alpha dipyridyl solution*.—Dissolve 0.1 gram of alpha-alpha dipyridyl (Eastman Co.) in water and dilute to 100 ml.

(c) *Hydroxylamine hydrochloride solution*.—Dissolve 10 grams of hydroxylamine hydrochloride in water and dilute to 100 ml.

Keep Reagents (a) and (b) in cool, dark place and they will remain stable for several weeks.

(d) *Magnesium nitrate solution*.—Dissolve 50 grams of  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  in water and dilute to 100 ml.

(e) *Acetate buffer solution*.—Dissolve 8.3 grams of C.P. anhydrous sodium acetate (previously dried at 100°C.) in water, add 12 ml. of glacial acetic acid, and dilute to 100 ml. (It may be necessary to redistil the C.P. glacial acetic acid and purify the sodium acetate by recrystallization from water, depending on amount of iron present.)

#### PREPARATION OF REFERENCE CURVE

(1) Dissolve 0.1 gram of analytical-grade iron wire in 20 ml. of HCl and 50 ml. of water, and dilute to 1 liter. Dilute 100 ml. of this solution to 1 liter. Each ml. has 0.01 mg. of iron. Or—

(2) Dissolve 3.512 grams of  $[(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}]$  in water, add 2 drops of HCl, and dilute to 500 ml. Dilute 10 ml. of this solution to 1 liter. Each ml. has 0.01 mg. of iron (Fe).

Make ten solutions with 2.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, and 45.0 ml., respectively, from the finally diluted stock solution. Also run a blank. Add 2.0 ml. of HCl and dilute to 100 ml. Use 10 ml. of each of these solutions and follow the procedure as given under "Determination," beginning "add 1 ml. of hydroxylamine solution." Plot concentration against scale reading.

#### DETERMINATION

Ash 10.0 grams of flour or air-dry bread in platinum, silica, or porcelain dish (about 60 mm. diameter, 35 ml. capacity) in accordance with method for ash, *Methods of Analysis*, A.O.A.C., 1940, 212, 5. (Porcelain evaporating dishes of approximately 25 ml. capacity are satisfactory. Do not use flat-bottomed dishes of greater diameter than 60 mm.) Cool, and weigh if percentage of ash is desired. Continue ashing until ash is practically carbon free. To diminish ashing time, or for samples that do not burn practically carbon free, use one of the following ash aids: Moisten the ash with 0.5–1.0 ml. of the  $\text{Mg}(\text{NO}_3)_2$  solution or with distilled  $\text{HNO}_3$ . Dry contents and carefully ignite in muffle to prevent spattering. (A white ash with no carbon results in most cases.) Do not add these ash aids to self-rising flour or bread (products containing salt) in a platinum dish because of vigorous action on the dish. Cool, add 5 ml. of HCl, evaporate to dryness on the steam bath, dissolve residue by adding, accurately measured, 2.0 ml. of HCl, heat for 5 minutes on the steam bath with watch-glass on the dish, wash off the watch-glass with distilled water, filter into a 100 ml. volumetric flask, cool, and dilute to volume. Pipet 10 ml. of aliquot into a 25 ml. volumetric flask, and add 1 ml. of the hydroxylamine solution; in a few minutes add 5 ml. of the buffer solution and 1 ml. of the orthophenanthroline or 2 ml. of the alpha-alpha dipyridyl solution and make to volume. Read the intensity of color

TABLE 5.—*Fe in iron solution, flour, whole wheat flour, bread, and self-rising flour*

COLLABORATOR	Fe SOLN	NO. 1	NO. 2	NO. 3	NO. 4
	mg./ml.	mg./lb.	mg./lb.	mg./lb.	mg./lb.
1	.01	6.7-6.7 Av. 6.7	16.4-16.9 Av. 16.6	14.8-14.0 Av. 14.4	9.5- 9.4 Av. 9.5
2	.01	7.1-6.9 Av. 7.0	15.9-15.9 Av. 15.9	13.4-13.6 Av. 13.5	9.4- 9.1 Av. 9.3
3	.01	7.3-6.9 Av. 7.1	16.8-16.6 Av. 16.7	13.4-13.4 Av. 13.4	9.4- 9.3 Av. 9.4
4	.0099	6.9-7.2 Av. 7.1	16.8-16.6 Av. 16.7	14.9	10.3-10.4 Av. 10.4
5	.01	7.5-7.5 8.0 Av. 7.7	17.4-17.4 17.6-18.5 Av. 17.7	14.7	12.7-10.0 10.2-10.4 Av. 10.8
6	.01	7.3-7.3 Av. 7.3	16.8-16.8 Av. 16.8	14.3-14.2 Av. 14.3	9.8- 9.7 Av. 9.8
7	—	7.7-7.7 Av. 7.7	17.2-17.2 Av. 17.2	14.1	9.9- 9.9 Av. 9.9
8	.01	Av. 7.3	Av. 17.3	Av. 14.5	Av. 9.5
9	.0103	7.6-7.2	16.9-16.8	14.3-14.5	Av. 9.7-10.4
Orthophenanthroline	.01	Av. 7.4	Av. 16.9	Av. 14.4	Av. 10.1
Alpha-alpha dipyridyl	.01	6.9-6.7 Av. 6.8	16.1-16.4 Av. 16.3	13.8-13.8 Av. 13.8	9.0-10.1 Av. 9.6
10	.0101	6.4-6.7 Av. 6.6	16.8-16.8 Av. 16.8	14.3-15.0 Av. 14.7	9.2- 9.5 Av. 9.4
11	.0097	6.2-6.0 Av. 6.1	16.3-16.6 Av. 16.5	13.8-13.9 Av. 13.9	9.3- 9.4 Av. 9.4
12	.011	6.4	17.3	14.5	10.2-10.0 Av. 10.1
13	—	6.9-7.1 7.6-6.9 6.8-6.4 Av. 7.0	16.8-16.8 16.8-17.2 16.7 Av. 16.9	14.3-14.5 14.4-14.3 14.4 Av. 14.4	9.2- 9.3 9.5- 9.5 9.5 Av. 9.4
14	—	6.8-6.8	16.9-16.9	13.8-13.8	10.1-10.2 Av. 10.2
Minimum		6.1	15.9	13.4	9.3
Maximum		7.7	17.7	14.7	10.8
Average		7.0	16.8	14.2	9.8



in a 2 inch cell on the neutral wedge photometer, using the No. 51 filter (about 514m $\mu$  wave length) or other suitable instrument of equivalent precision. From the reading, determine the concentration of iron from the equation of the line representing the standard points or by reference to standard curve for known iron concentration. Determine blank on the reagents and make correction. Calculate amount of iron in the flour or bread as mg. per pound. Rinse all flasks, beakers, funnels, etc., with distilled water before use, and filter all reagents to remove suspended matter.

The results presented in Table 5 were obtained from 14 collaborators.

The results in Table 5 indicate the range to be expected on samples of similar material by different chemists. These variations are within a reasonable degree of agreement when compared with the average for the group and the amount of the iron calculated in No. 1 and No. 4 of 6.9 and 9.7 mg./lb., respectively. The excellent agreement on the iron solution indicates that only a small part of the variation on the samples is caused by the measurement of the concentration of the iron in solution.

#### SUMMARY

This report describes a simple and convenient method for the determination of iron and indicates the degree of accuracy and agreement to be expected by different analysts on the products used.

#### CALCIUM

The same samples sent out for collaborative study on iron were used for calcium determination. Samples No. 1 and 4 contained 450 and 1505 mg./lb. of calcium based on calculation from analyses of ingredients. The calcium content was not known on Nos. 2 and 3. The collaborators were instructed to use 50 ml. aliquot of the solution from the iron determination, dilute to about 200 ml. and proceed as directed in *Methods of Analysis*, A.O.A.C., 1940, 339, 19, as far as "and allow mixture." Then to proceed as directed under 48, p. 366, beginning, "let stand overnight except for titration with 0.05 N KMnO<sub>4</sub>. Calculate Ca as mg./lb."

The results are shown in Table 6.

The maximum value for Sample 3 and the maximum and minimum values for Sample 4 seem considerably at variance with the other results and have been omitted from the average. Reasonable agreement was obtained on the other results as compared with the average and calculated content of calcium in Sample 1 of 450 mg./lb. and Sample 4 of 1505 mg./lb.

It is recommended\*—

(1) That the procedures given in this report for the determination of iron and calcium be adopted as tentative.

(2) That the study be continued.

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\* For report of Subcommittee D and action by the Association see *This Journal* 27, 66, 86 (1944).

TABLE 6.—*Calcium in flour, whole wheat flour, bread, and self-rising flour*

COLLABORATORS	NO. 1	NO. 2	NO. 3	NO. 4
	<i>mg./lb.</i>	<i>mg./lb.</i>	<i>mg./lb.</i>	<i>mg./lb.</i>
1	431-429 Av. 430		540-544 Av. 542	1502-1509 Av. 1506
2	438-440 Av. 439	232-234 Av. 231	527-531 Av. 529	1471-1494 Av. 1483
3	430-426 Av. 428	237-240 Av. 239	539-528 Av. 534	1487-1466 Av. 1477
4	398-391 Av. 395	221-215 Av. 218	500	1328*
5	460-474-466 Av. 467		661	1566-1526-1500 Av. 1531
6	459-449 Av. 454		579-569 Av. 574	1537-1527 Av. 1532
7	452-464 Av. 458		554	1509-1509
8	431	232	540	1516
9	412-411 Av. 412	219-213 Av. 216	550-535 Av. 543	1472-1494 Av. 1483
10	443-443	266-248 Av. 257	522-527 Av. 525	1505-1505
11	425-434 Av. 430		539-534 Av. 537	1498-1507 Av. 1503
12	408-417 Av. 413	238-225 Av. 232	539-506 Av. 523	1471-1489 Av. 1480
13	454-454- 472-454 Av. 457	232-244 Av. 238	526-545 Av. 536	1570-1560 Av. 1565
14†	472	257	540-532 Av. 536	2214-2184 Av. 2199*
Minimum	395	216	500	1328*
Maximum	472	267	661*	2199*
Average	438	237	536	1505

\* Omitted from average.

† Collaborator 14, due to an error in calculation, has since reported the results on No. 4 should have been 1510 and 1489, Av. 1500 mg./lb.

The splendid cooperation of the following collaborators is greatly appreciated:

A. G. Buell, C. C. Cooley, S. Alfend, G. M. Johnson, L. E. Wener, F. J. McNall, I. Schurman, L. W. Ferris, M. Tubis, C. A. Wood, all of the Food and Drug Administration; John S. Andrews, of General Mills, Inc.; C. G. Harrel, of Pillsbury Flour Company; and W. H. King of Department of Health, New Orleans.

## REPORT ON STARCH IN RAW AND BAKED CEREALS

By M. P. ETHEREDGE (Mississippi State Chemical Laboratory, State College, Miss.), *Associate Referee*

For the past two years the Hopkins revision of the Mannich-Lenz procedure<sup>1</sup> for the determination of starch in cereals has been studied by the Association and compared, principally, with the Rask procedure (*Methods of Analysis, A.O.A.C.*, 1940, 221). In 1942, some comparisons were also made with the diastase-hydrochloric acid procedure (*Ibid.*, 359) and reported in *This Journal*, 26, 214. Owing to increased interest in starch determinations, reflected by eight collaborators participating, it was decided to make further studies this year and to compare the polarimetric method with the diastase-hydrochloric acid procedure.

The following samples were selected for study: No. 1, ground whole corn; No. 2, white bread; No. 3, cornstarch; No. 4, wheat starch.

The corn was yellow corn (Grade No. 2) prepared by J. H. Shollenberger of the Northern Regional Research Laboratory, Peoria, at the suggestion of R. T. Milner, Chief, Analytical and Physical Chemical Division, of the same laboratory. The white bread was baked by a local bakery in Starkville, Miss. Bread from this same bakery was used in the report of 1941 (*This Journal*, 25, 621). The Associate Referee obtained 59.66 per cent starch in 1941, and by the same method he is reporting 60.04 this year. The cornstarch sample, furnished by J. P. Bishop of Corn Products Refining Company, Argo, Illinois, is their No. 126 starch, without further purification. The wheat starch, furnished by Stein, Hall and Company of New York, is a powdered commercial grade without further treatment. All samples were passed through a 100-mesh sieve and were allowed to come to a moisture equilibrium in the Mississippi State Chemical Laboratory before being sent out for analysis.

The samples were sent to fifteen collaborators with instructions to test by both the Hopkins and the diastase-hydrochloric acid procedures. With the Hopkins method, it was advised in case of difficulty with filtering that the analyst centrifuge with Celite, as suggested by J. D. Guthrie and E. T. Steiner of the Southern Regional Laboratory, New Orleans. As specific

<sup>1</sup> *Can. J. Research*, 11, 751-758 (1934).

TABLE 1.—*Starch in corn and wheat products*

ANALYST	GROUND WHOLE CORN		WHITE BREAD	
	HOPKINS POLARIMETRIC METHOD	DIASTASE- HYDROCHLORIC ACID METHOD	HOPKINS POLARIMETRIC METHOD	DIASTASE- HYDROCHLORIC ACID METHOD
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	65.10	59.90	60.30	57.40
	65.40	60.50	60.10	57.10
	64.80	60.40		57.40
		59.70		
Av.	65.10	60.13	60.20	57.30
2	65.00	61.50	60.40	58.30
	64.90	60.60	60.40	57.60
	64.80	60.60	60.30	58.90
Av.	64.90	60.90	60.37	58.27
3	65.30	64.80	60.48	61.79
	65.24	64.33	60.45	62.12
	65.33	64.18	60.09	61.95
	65.26	64.27	60.29	61.40
Av.	65.28	64.40	60.33	61.82
4	64.57		57.59	
	64.79		58.37	
Av.	64.68		57.98	
5	64.66	62.57	60.39	60.15
6	64.40	57.69	55.75	54.96
	64.50	58.07	55.25	54.15
		58.97		53.88
Av.	64.45	58.24	55.50	54.33
7	64.84	59.90	61.38	56.20
	64.84	60.80	61.81	56.50
	65.70	59.70	60.52	56.60
	64.84	58.80	61.81	56.60
Av.	65.06	59.80	61.51	56.48
8	65.30	61.20	60.70	58.20
	65.30	61.30	60.90	58.30
	65.30	61.30	60.90	58.10
		61.50		58.50
Av.	65.30	61.33	60.83	58.28

TABLE 1.—*Continued*

ANALYST	GROUND WHOLE CORN		WHITE BREAD	
	HOPKINS POLARIMETRIC METHOD	DIASTASE- HYDROCHLORIC ACID METHOD	HOPKINS POLARIMETRIC METHOD	DIASTASE- HYDROCHLORIC ACID METHOD
9	<i>per cent</i> 67.20	<i>per cent</i> 64.26	<i>per cent</i> 66.50	<i>per cent</i> 64.60
	68.00			
Av.	67.60	64.26	66.50	64.60
10	63.75	59.52	59.95	57.39
	63.80	60.67	60.12	57.92
	63.67			
	63.71			
Av.	63.73	60.10	60.04	57.66
*11	65.60		60.40	
	65.60		60.40	
	65.40		60.80	
	65.40		59.20	
Av.	65.50		60.20	
*12	63.40	61.70	59.40	57.94
	63.60	62.10	59.60	58.38
	63.60		59.60	58.67
Av.	63.53	61.90	59.53	58.33
* 3 (corrected)		63.12		60.18
		62.66		60.50
		62.51		60.34
		62.60		59.80
Av.		62.72		60.21

\* Too late to be included in averages and discussions.

rotation, 200 was to be used for all starches. The variations suggested for the diastase-hydrochloric acid procedure were: (1) to wash with 70 per cent alcohol instead of 10 per cent; (2) to use any enzyme desired; (3) to use toluene or thymol during hydrolysis to keep down microbiological action (suggested by K. A. Clendenning of National Research Council, Ottawa); and (4) to buffer at pH 4.5–6.0 (suggested by R. T. Milner and R. W. VonKorff of the Northern Regional Laboratory, Peoria). As the dextrose-starch factor, 0.9 was to be used.

TABLE 2.—*Starch in two commercial starches*

ANALYST	CORNSTARCH		WHEAT STARCH	
	HOPKINS POLARIMETRIC METHOD	DIASTASE- HYDROCHLORIC ACID METHOD	HOPKINS POLARIMETRIC METHOD	DIASTASE- HYDROCHLORIC ACID METHOD
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	88.10	81.70	86.80	81.50
	87.90	81.60	86.60	81.20
	88.60	82.60	87.10	81.50
	87.80			80.90
Av.	88.10	81.97	86.80	81.28
2	88.20	84.00	87.10	83.00
	88.00	83.80	87.10	82.40
	88.10	83.90	87.10	82.70
Av.	88.10	83.90	87.10	82.70
3	87.63	86.84	86.66	85.89
	87.80	87.04	86.55	85.03
	87.55	87.83	86.91	85.87
	87.79	87.74	86.95	84.97
Av.	87.69	87.37	86.77	85.44
4	88.70		87.44	
	88.68		87.59	
Av.	88.69		87.52	
5	87.36	83.96	86.45	83.15
6	90.88	79.70	89.02	77.80
	89.80	80.39	88.38	77.49
	90.50	79.63	89.45	
Av.	90.39	79.91	88.95	77.49
7	89.04	81.00	87.31	79.30
	88.18	80.70	87.75	79.90
	88.18	81.10	88.18	79.30
	88.61	79.70	87.75	79.10
Av.	88.50	80.60	87.75	79.40
8	86.90	83.70	87.20	81.30
	86.90	83.70	87.20	81.00
	86.50	84.00	87.20	81.30
		83.60		81.00
Av.	86.78	83.75	87.20	81.15

TABLE 2.—Continued

ANALYST	CORN STARCH		WHEAT STARCH	
	HOPKINS POLARIMETRIC METHOD	DIASTASE- HYDROCHLORIC ACID METHOD	HOPKINS POLARIMETRIC METHOD	DIASTASE- HYDROCHLORIC ACID METHOD
9	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	89.10	86.04	87.04	83.88
	89.50			
Av.	89.30	86.04	87.04	83.88
10	86.61	83.84	85.91	83.84
	85.74	84.69	86.04	83.84
	86.79		86.34	
	87.06		86.43	
Av.	86.80	84.27	86.18	83.84
*11	87.90		87.00	
	87.90		87.00	
	87.80		87.00	
	87.90		87.00	
Av.	87.88		87.00	
*12	87.25	82.47	85.75	82.76
	87.38	82.57	85.83	82.80
	87.50		86.00	83.47
				83.43
Av.	87.38	82.52	85.86	83.10
* 3 (corrected)		83.19		82.28
		83.38		81.46
		84.14		82.26
		84.05		81.40
Av.		83.69		81.85

\* Too late to be included in averages and discussions.

Results were received from nine collaborators, whose efforts make this report possible. The Associate Referee deeply appreciates this cooperation during the present period of severe strain. The complete list of collaborators follows and their results are shown in the tables.

#### COLLABORATORS

V. E. Munsey, Food and Drug Administration, Washington.

R. T. Milner and Mrs. R. M. Glassco, Northern Regional Research Laboratory, Peoria.

J. D. Guthrie and E. T. Steiner, Southern Regional Research Laboratory, New Orleans.

C. Y. Hopkins and Miss M. Chisholm, National Research Council, Ottawa.

The W. E. Long Company, Chicago.

Betty L. Geiger, General Mills, Minneapolis.

G. T. Peckham, Jr., Clinton Company, Clinton, Iowa.

H. File and E. K. Olson, A. E. Staley Manufacturing Company, Decatur, Ill.

H. H. Schopmeyer, American Maize-Products Company, Roby, Ind.

M. P. Etheredge.

\* K. A. Clendenning, National Research Council, Ottawa.

\* C. O. Willits and W. L. Porter, Eastern Regional Research Laboratory, Philadelphia.

## DISCUSSION OF RESULTS

If an analyst gave more than four results, the wider ones were discarded. Unfortunately, some gave only one result, presumably an average. No analyst checked himself better by the diastase-hydrochloric acid method than by the Hopkins procedure. Only two analysts checked themselves as well by the diastase-hydrochloric acid process, and one of these expressed a preference for the Hopkins procedure.

Although arithmetical, or accepted, averages have not been computed, it is interesting to look at the averages and compare the two methods as to spread between analysts. With the corn sample, if the high and the low results be eliminated there would be only 0.85 per cent total spread by the Hopkins procedure, while there would still be 4.46 between the high and low averages by the diastase-hydrochloric acid procedure. With the bread sample it would be necessary to cut out two results, each high and low, to give only 0.76 spread by the Hopkins procedure, while a like process of elimination would leave 2.85 difference by the other method. A similar process of elimination in the averages of the corn and wheat starches leaves 1.00 versus 2.27 and 0.75 versus 2.64, respectively.

While such variations among the analysts are regretted, they sometimes occur in well worked-out methods. Some of the analysts who participated this year had never had experience with the determination of starch. However, it is definitely noticeable that the variations of analysts by the diastase-hydrochloric acid method are more than double the variations by the Hopkins procedure. Also, in the case of the commercial starches, the Hopkins procedure gives a more accurate value of starch.

It is believed that all analysts used some form of malt diastase as enzyme in the diastatic conversion of the previously mentioned results. However, Referee Munsey also tried out takadiastase, polydase, and mylase. In most instances, the conversion was not so satisfactory. This seems to be the opinion of other collaborators who have had experience with other enzymes. Munsey also obtained slightly higher results by treating with diastase three times instead of twice. The Associate Referee has

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\* Too late to be included in averages and remarks.



found a similar increase by treating for longer periods and with larger amounts of diastase.

#### OTHER METHODS

Referee Munsey, and H. H. Schopmeyer of the American Maize-Products Company, tried all four samples by direct acid hydrolysis, and both analysts showed results too high on the corn, as would be expected; on the other samples one obtained higher results and the other lower without first treating with diastase. The lower results are not understood. The Associate Referee reported similar experiences with starches to the Association in 1940 (*This Journal*, 24, 113).

Schopmeyer also tried all four samples by the American Maize-Products Company persulfate method (private communication). The results were not consistent when compared with results from other methods. This method is said to be used for determining starch in fibrous material, and it would not be generally recommended for products having high starch content.

The Associate Referee did not try out the Clendenning method<sup>2</sup> referred to last year. It directs that the proteins be precipitated with stannic chloride. This method seems to be specific for flours, and no flour sample was sent out this year.

In his search for a better dispersion agent the Associate Referee tried out another group of chemicals, but unfortunately, was again unsuccessful. This list of chemicals follows: o-nitrobenzoic, o-benzoylbenzoic, phenyl salicylate, cadmium nitrate, sodium nitrate, potassium acid oxalate, cream of tartar, cerium oxalate, paraldehyde, eucalyptus, p-hydroxybenzoic, succinic, naphthalic anhydride, phthalic anhydride, and cellulose acetate.

#### ADDITIONAL WORK

The Association has realized for some time that the glucose-starch factor of 0.9 is too low for all practical purposes, and also that the specific rotation of 200 for most starches is too low. With this in mind, the Associate Referee requested the collaborators to endeavor to arrive at a better factor for the starch and wheat samples sent out. Only two collaborators found time to work on this project, and they are in wide disagreement. The work done by the Associate Referee basically agrees with one of them, but here, too, there is a small difference due to a variation in moisture determination. It seems that any approach to a specific rotation factor or to a glucose-starch factor is based on getting starch by difference. This involves a moisture determination, which is usually not made with a high degree of accuracy. It was thought best not to report the small amount of work done on factors until more data can be accumulated. However, as each analyst reported results on moisture on the samples they are listed in Table 3.

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<sup>2</sup> *Can. J. Research*, 20, 403 (1942).

TABLE 3.—*Moisture in samples*

ANALYST	METHOD	CORN	BREAD	CORNSTARCH	WHEAT STARCH
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	100° vacuo	11.40	11.37	12.12	11.91
2	105° vacuo—20 hrs.	12.26	11.68	12.34	12.62
3	100° vacuo—16 hrs.	11.96	11.50	12.44	12.45
4	105° vacuo—5 hrs.	10.60	10.76	11.62	11.91
5		10.74	11.21	12.02	12.12
6	130° air—1 hr.	10.40	11.00	12.00	12.00
7	115° air—1 hr.	11.00	10.80	11.10	11.40
8	100° vacuo	10.98	10.63	11.43	11.69
9		11.10	10.30	10.40	12.60
10	105° air—20 hrs.	11.02	10.94	11.68	11.92

## DISCUSSION OF MOISTURE RESULTS

No two analysts agree well on all four samples. The nearest approach to an agreement is by Analysts 2 and 3. They both used long hours in vacuo. R. T. Milner, Northern Regional Laboratory, Peoria, seems to have had more experience with moisture in starch than most collaborators. He has contributed some interesting comparisons between the toluene (Bidwell-Sterling) method and drying in vacuo at 120° for 24 hours. The results are given in Table 4.

TABLE 4.—*Moisture in starch*

METHOD	NO. 59	NO. 60	NO. 61	NO. 62
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Toluene	11.67	12.10	12.02	12.12
Vacuum oven	11.58	12.03	11.96	11.96

In view of the fact that the toluene distillation method has always been regarded as the most accurate for this type of moisture, it must be concluded after looking at Tables 3 and 4, that a long drying period in vacuo is necessary. Sair and Fetzger give more details on this subject in a recent report.<sup>3</sup>

## CONCLUSIONS

The work of the past two years combined with that of this year indicates quite plainly that the Hopkins revision of the Mannich-Lenz procedure for quantitative estimation of starch is comparable with, if not superior to, any known method. It is believed that further work of the Association should include a careful study of specific rotation. The Associate Referee considers that calcium chloride brine is not an ideal dispersion medium. However, if a better medium can not be found and the As-

<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 14, 843 (1942).

sociation can arrive at a fairly constant rotation figure, then this method should receive serious consideration for adoption as a tentative method. Also, the glucose-starch factor of 0.9 is too low, and it should be correspondingly revised upwards.

The Hopkins revision of the Mannich-Lenz procedure for the determination of starch was adopted as a tentative method. The details of this method follow:

#### STARCH<sup>1</sup>

##### REAGENT

*Calcium chloride solution.*—Dissolve 2 parts of crystalline  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  in one part of water and adjust to density of 1.30 at 20°. (This solution contains about 33% of  $\text{CaCl}_2$ .) Make faintly pink to phenolphthalein by adding 0.1 N NaOH. (Anhydrous  $\text{CaCl}_2$  may be used, but it is usually alkaline and requires the addition of acid to bring it to correct pH.)

##### DETERMINATION

Grind sample finely (100-mesh if possible) and weigh 2.0–2.5 grams into a 50 ml. round-bottomed centrifuge tube with lip. Wash with ether to remove fat, then with 10 ml. of approximately 65% by weight aqueous alcohol ( $d_{20}$  0.88) and stir thoroughly with a glass rod. Centrifuge (1) and pour off the solution. Repeat the washing until 60 ml. of wash liquid has been used, stirring each time with same rod.

Stir residue with 10 ml. of water and pour into a 200–250 ml. Erlenmeyer flask. Complete transfer by washing out with a total of 60 ml. of the  $\text{CaCl}_2$  solution containing 2 ml. of 0.8% acetic acid. Transfer rod to flask and bring mixture to boiling quickly over wire gauze, stirring frequently. Boil briskly for 15–17 minutes, taking precautions to prevent burning and foaming (2). Rub the particles on sides of flask down with the rod from time to time.

Cool solution quickly in running water and pour into a 100 ml. volumetric flask, rinsing thoroughly with the  $\text{CaCl}_2$  solution from a wash-bottle with a medium jet. (In making up to mark, it is permissible to destroy the froth by adding one drop of alcohol.)

After thoroughly mixing the sample pour about 10 ml. of the solution onto a fluted filter (Whatman No. 42 or 44), wetting paper completely. Allow filtrate to run dry and discard. Resume filtration, using a dry receiver, and collect 40–50 ml. (3).

Polarize the liquid in a 10 cm. tube, taking 2 sets of 10 readings each. (The averages of the two sets should agree within 0.006°.) Calculate the starch content as follows:

$$\text{Percentage starch (4)} = \frac{100 \times A \times 100}{1 \times 200 \times S} = \frac{50 \times A}{S}, \text{ where } A \text{ is observed rotation and}$$

$S$  is weight of sample.

#### NOTES:

(1) If no centrifuge is available wash samples on a filter paper, using a Pt cone and slight suction.

(2) An Argand burner with a thin asbestos gauze will diminish foaming.

(3) For filtering aids, Celite with Pyrex glass filters and Hirsch-type funnel with asbestos and suction are recommended.

(4) 200 is arbitrarily taken as specific rotation for all starches until a better figure is worked out for individual starches. If a 200 mm. tube and a saccharimeter are used, 2 grams of sample is weighed out and the mixture is made up to 100 ml, each °V will be multiplied by 4.3225 to give % of starch.

<sup>1</sup> *This Journal*, 10, 108; 11, 484; 12, 890; 13, 447; 14, 112; 15, 583; 16, 505; 17, 400; 18, 870; 19, 621; 20, 547; 21, 360, 394; 22, 523; 23, 489; 24, 113.

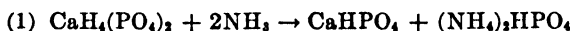
## CONTRIBUTED PAPERS

### FACTORS THAT INFLUENCE THE $P_2O_5$ TRANSITIONS THAT OCCUR IN AMMONIATION OF SUPERPHOSPHATE

By W. H. MACINTIRE, H. L. MARSHALL, and R. C. SHANK  
(The University of Tennessee Agricultural Experiment  
Station and Department of Chemical Engineering,  
Tennessee Valley Authority, Knoxville, Tenn.)

In his pioneer investigations, Keenen (12) observed that the basic phosphates formed in the ammoniation of superphosphate were decidedly less soluble than the corresponding tertiary precipitates of reagent grade. The nature and properties of the water-insoluble phosphates induced by ammoniation of superphosphates are affected by the impurities carried by the starting rock phosphate, but analogous tertiary calcium phosphates of reagent grade also vary markedly in composition and reactivity, in structure, and in fertilizer value (9, 11, 18, 20).

Brioux (4) and Gerlach (5) had shown that full ammoniation of superphosphate results in the development of tricalcium phosphate and ammonium sulfate according to the equation,  $CaH_4(PO_4)_2 + 2CaSO_4 + 4NH_3 \rightarrow Ca_3(PO_4)_2 + 2(NH_4)_2SO_4$ . Keenen (12), Keenen and Morgan (13), and Harford and Keenen (8) stressed the instability of generated diammonium phosphate as a causal factor in the  $P_2O_5$  transitions that occur, as indicated by the equations:



In practice, however, it soon was found that even partial ammoniation of superphosphate may induce the formation of phosphates less soluble than normal tricalcium phosphate, and Andrews (1) has demonstrated that this distinction in solubility is registered also by plant response. Jacob and Ross (10), Hardesty and Ross (6, 7), While, Hardesty, and Ross (21), and Marshall, Hendricks, and Hill (19) concluded that decrease in chemically evaluated "availability" is caused by the generation of "hydrated" forms.

The fact that concentrated superphosphate can be ammoniated without serious degradation in  $P_2O_5$  availability has been attributed to low content of calcium sulfate (12, 16). Bassett (2) pointed out, however, that at 80°C. the conversion of dicalcium phosphate will occur in the absence of calcium sulfate, according to the equation,  $3CaHPO_4 + 2NH_3 \rightarrow Ca_3(PO_4)_2 + (NH_4)_2HPO_4$ .

It also has been demonstrated that component fluorides react with certain forms of tertiary calcium phosphates in either ammoniated or limed superphosphates with resultant formation of calcium fluorophosphate (14,

16, 17), whereas such reversion was virtually absent in ammoniated and in limed superphosphates derived from defluorinated rock phosphate (16). The phenomenon of fluorophosphate formation likewise accounts for the retrogradation that occurred when superphosphate was fortified with defluorinated calcines of rock phosphate (3), although reversion did not occur when defluorinated quenched fusions of rock phosphate were admixed with superphosphate (15). Recently, it has been found that hydroxyphosphate reacts with fluorides much more readily than does the normal tertiary (18). Hence, the greater the quantity of hydroxyphosphate generated in ammoniated superphosphates the greater will be the subsequent formation of fluorophosphate.

#### OBJECTIVE

The present study deals with proportions of reactants, temperature, additive calcium sulfate of variant degree of hydration, aging, component fluorides, and variance in technic of citrate digestion as governing factors in establishing the transitions induced by gaseous and aqueous ammoniations of superphosphates.

#### EXPERIMENTAL

Since the ammoniation of the superphosphate complex is complicated by several possible vitiative reactions, reagent grades of mono- and dicalcium phosphates and different forms of calcium sulfate were used in a series of exploratory ammoniations. The several reactants of the 150 ml. aqueous systems of Tables 1 and 2 were introduced in the indicated molal proportions in closed containers at room temperature. The several systems were agitated vigorously by hand and then were maintained quiescent at temperatures of 30° and 90°C. for durations of 24, 72, and 144 hours. The solutions and the solids of the experimental systems were separated and analyzed at those periods and the phases of the systems were computed.

The solubility of the tertiary formed at 30°C., with concomitant diammonium phosphate, in the 8-mol ammoniations of System 1, indicated a normal tricalcium phosphate. The precipitate was least soluble at the 144-hour period. The corresponding precipitate formed at 90°C. was considerably less soluble and its  $P_2O_5:CaO$  ratio was lower and closer to that of hydroxyapatite. Although the triplicate precipitates formed in the 90°C. systems were identical as to ratio, the 144-hour precipitate proved least soluble.

In the replicates of System 2 at 30°C., the precipitates were dicalcium phosphate of constant  $P_2O_5:CaO$  ratio and were stable in the solution of generated monoammonium phosphate. The dicalcium phosphate formed in the parallel system at 90°C. was slightly less soluble, however, and solubility was depressed somewhat by the hydrolysis indicated by decrease in the concentration of the monoammonium solute during aging.

In System 3 the dicalcium phosphate formed in the presence of calcium sulfate at 30°C., with the concomitant diammonium phosphate solute,

TABLE 1.—*Ammoniation of monocalcium phosphate in aqueous systems, with and without CaSO<sub>4</sub>, at two temperatures*

SYSTEM NUMBER	AMMONIATED SYSTEM		DIGESTION	NITROGEN CONTENT <sup>a</sup>	SOLUTE			INDICATED		
	REACTANTS	RESULTS			°C.	per cent	SOLUTE		INDICATED	
							(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(NH <sub>4</sub> )H <sub>2</sub> PO <sub>4</sub>	CaHPO <sub>4</sub>	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>
			hours			P <sub>2</sub> O <sub>5</sub> /NH <sub>3</sub>	P <sub>2</sub> O <sub>5</sub> /NH <sub>3</sub>	P <sub>2</sub> O <sub>5</sub> /CaO	P <sub>2</sub> O <sub>5</sub> /CaO	% of T
(1)	3CaH <sub>2</sub> (PO <sub>4</sub> ) <sub>2</sub> , 8NH <sub>3</sub>	4(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> , Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	24	30	15.2	absent	2.069	absent	0.827	59.8
			72	30			2.145		0.885	59.1
			144	30			2.019		0.839	54.1
			24	90	15.2	absent	2.255	absent	0.804	43.3
			72	90			2.260		0.804	43.8
			144	90			2.221		0.804	38.6
(2)	CaH <sub>2</sub> (PO <sub>4</sub> ) <sub>2</sub> , NH <sub>3</sub>	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> , CaHPO <sub>4</sub>	24	30	6.3	4.747	absent	1.263	absent	99.9
			72	30		4.810		1.248		99.9
			144	30		4.736		1.238		99.9
			24	90	6.3	4.341	absent	1.246	absent	98.6
			72	90		4.402		1.263		96.2
			144	90		4.427		1.243		96.1
(3)	2CaH <sub>2</sub> (PO <sub>4</sub> ) <sub>2</sub> , 4NH <sub>3</sub> , CaSO <sub>4</sub>	2(NH <sub>4</sub> ) <sub>2</sub> H <sub>2</sub> PO <sub>4</sub> , (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	24	30	9.8	absent	2.451	1.252	absent	100.0
			72	30		2.529	1.211	99.8		
			144	30		2.497	1.237	99.9		
			24	90	9.8	3.412	absent	absent	0.983	59.3
			72	90		3.551		56.0		
			144	90		3.742		0.977	52.6	
(4)	CaH <sub>2</sub> (PO <sub>4</sub> ) <sub>2</sub> , 4NH <sub>3</sub> , 2CaSO <sub>4</sub>	2(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	24	30	11.5	absent	absent	absent	0.977	51.0
			72	30				0.940	50.0	
			144	30				0.821	45.9	
			24	90	11.5	absent	absent	absent	0.637	68.4
			72	90				0.668	73.1	
			144	90				0.658	74.1	
(5)	2CaH <sub>2</sub> (PO <sub>4</sub> ) <sub>2</sub> , 3NH <sub>3</sub> , and CaSO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> H <sub>2</sub> PO <sub>4</sub> , (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , 3CaHPO <sub>4</sub>	24	90	2.7	4.173	absent	1.225	absent	99.4
			72	90		4.359		1.244		99.5
			144	90		5.131		1.231		99.4
	and CaSO <sub>4</sub> ·1/2H <sub>2</sub> O and CaSO <sub>4</sub> ·2H <sub>2</sub> O		24	90	2.7	4.157	absent	1.226	absent	99.8
			72	90		4.288		1.233		99.8
			144	90		4.248		1.232		99.8
	and CaSO <sub>4</sub> ·2H <sub>2</sub> O		24	90	2.6	4.129	absent	1.226	absent	99.6
			72	90		4.381		1.227		99.4
			144	90		5.106		1.262		99.7

<sup>a</sup> Expressed as percentage of potential salt content of the systems.  
<sup>b</sup> By 1-hour continuously agitated extractions with neutral ammonium citrate of 1.09 sp. gr.

TABLE 2.—Ammoniation of dicalcium phosphate in aqueous systems, with and without  $\text{CaSO}_4$ , at two temperatures

SYSTEM NUMBER	AMMONIATED SYSTEM		DIGESTION	NITROGEN CONTENT <sup>a</sup>	INDICATED			
	REACTANTS	REACTANTS			SOLUTE		SOLID	AVAIL. $\text{P}_2\text{O}_5^b$
			hours	Per Cent	$(\text{NH}_4)_2\text{HPO}_4$	$(\text{NH}_4)_2\text{HPO}_4$	$\text{Ca}_3(\text{PO}_4)_2$	
(1)	$3\text{CaHPO}_4$ $2\text{NH}_3$	$(\text{NH}_4)_2\text{HPO}_4$ $\text{Ca}_3(\text{PO}_4)_2$	24	30		$\text{P}_2\text{O}_5/\text{NH}_3$	$\text{P}_2\text{O}_5/\text{CaO}$	% of T
			72	30	absent	0.636	1.077	80.4
			144	30		1.412	0.932	53.3
						1.726	0.903	46.2
			24	90	2.909		0.886	27.7
			72	90	4.095	absent	0.980	51.7
(2)	$2\text{CaHPO}_4$ $2\text{NH}_3$ $\text{CaSO}_4$	$(\text{NH}_4)_2\text{SO}_4$ $\text{Ca}_3(\text{PO}_4)_2$	144	90	4.226		1.119	59.4
			24	30			0.981	81.6
			72	30			0.930	57.6
			144	30			0.835	52.8
			24	90			0.869	22.3
			72	90			0.853	24.4
			144	90			0.830	26.3

<sup>a</sup> Expressed as percentage of potential salt content of the systems.<sup>b</sup> By 1-hour continuously agitated extractions with neutral ammonium citrate of 1.09 sp. gr.

was completely citrate-soluble at each period. At 90°C., however, the unstable diammonium phosphate solute was converted to the mono form in the conversion of the dicalcium precipitate to one of tricalcium phosphate, the citrate solubility of which decreased with aging.

In System 4, of greater calcium sulfate proportion, and no final  $\text{PO}_4$  solute, the tertiary generated at 30°C. showed a progressive decrease in  $\text{P}_2\text{O}_5$  proportion and in citrate-solubility. The triplicate precipitates formed at 90°C. were of uniform  $\text{P}_2\text{O}_5$ :CaO ratio at the three periods, and were more soluble than those formed in the analogous units of System 3.

In the nine units of System 5, all maintained at 90°C., the reactants were identical except for the variation as to the hydration of the additive sulfate. In all of these systems the 1:3:1 starting ratio of monocalcium phosphate, ammonia, and calcium sulfate induced precipitates of dicalcium phosphate of uniform composition and virtually complete citrate solubility. Variation in the form of the calcium sulfate exerted no effect upon the dicalcium phosphate formation in these nine systems that contained ammonia, calcium sulfate, and monoammonium phosphate.

In the systems of Table 2, suspensions of dicalcium phosphate, instead of the monocalcium salt, were used with and without calcium sulfate. The effect of time upon change in the solubility of the generated tertiary was downward in the first units of System 1 and upward in the three corresponding units maintained at 90°C. Coincident with the progressive decrease in the solubility of the tertiary formed in the 30°C. units, there occurred an increase in the concentration of solute diammonium phosphate. In contrast, the progressive increase in the solubilities of the tertiary formed in the 90°C. systems was integrated with the progressive increase in the concentration of the generated monoammonium phosphate. Hence, after 144 hours the precipitate formed at 90°C. was more soluble than the corresponding precipitate formed at 30°C. This registers the progression in the reformation of dicalcium phosphate from the initially generated tertiary and the disappearance of solute diammonium phosphate.

In the second series of Table 2 the progressive decrease in the solubility of the generated tertiary upon aging at 30°C. reflects the early completeness of the transition of the dicalcium phosphate to hydroxyapatite, but with illogical indication of transition to the normal phosphate ratio for the least degree of solubility. At the higher temperature, immediate formation of the hydroxy form of low solubility was registered. Upon aging, however, the tertiary precipitate became more soluble as the result of a decrease in its calcium content through the dissolvent action of the built up concentration of ammonium sulfate.

#### EFFECT OF VARIATION IN CITRATE DIGESTIONS UPON SOLUBILITIES OF AGED TERTIARIES OF TABLES 1 AND 2

The variances in composition of the tertiaries obtained after 144 hours at the two temperatures of Systems 1, 3, and 4 of Table 1 and the two sys-



tems of Table 2 were explored by the variations in the technic of citrate digestions reported in Table 3. In seven of the nine comparisons, continuous agitation gave solubilities higher than those obtained by agitation at 5-minute intervals. In every instance, a substantial enhancement in citrate-soluble value was registered by the continuously agitated digestion of the residue from the single digestion. The mean of the enhancements was 45 per cent. The precipitates formed at 30°C. in the no-sulfate units

TABLE 3.—*Effect of variation in citrate digestions upon solubilities of the aged tertiaries of Tables 1 and 2*

			P <sub>2</sub> O <sub>5</sub>										
SYSTEM			P <sub>2</sub> O <sub>5</sub> /CaO OF TERTIARIES	CITRATE-INSOLUBLE							CITRATE-SOLUBLE		
NO.	TABLE	TEMP.		TOTAL	PERIODIC AGITATION, <sup>a</sup>		CONTINUOUS AGITATION <sup>b</sup>			PERIODIC AGITATION <sup>a</sup>		CONTINUOUS AGITATION <sup>b</sup>	
					SINGLE 1:100	DOUBLE 1:100	SINGLE 1:100	DOUBLE 1:100	SINGLE 1:100	SINGLE 1:100	DOUBLE <sup>c</sup> 1:100		
°C.			per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent		
1	1	30	0.839	42.5	19.5	16.4	4.0	54.1	61.5	90.6			
1	1	90	0.804	42.2	25.9	24.4	8.8	38.6	42.2	79.1			
3	1	90	0.977	46.0	21.8	21.1	13.8	52.8	54.1	70.0			
4	1	30	0.821	41.8	22.6	20.1	5.6	45.9	51.9	86.6			
4	1	90	0.658	31.6	8.2	8.4	0.75	74.1	73.4	97.6			
1	2	30	0.903	44.8	24.1	23.4	12.4	46.2	47.8	72.3			
1	2	90	1.119	48.0	19.5	18.3	14.3	59.4	61.9	70.2			
2	2	30	0.835	40.3	21.3	21.3	9.6	52.8	52.8	76.2			
2	2	90	0.830	41.5	26.3	26.0	17.8	36.6	37.4	57.1			

<sup>a</sup> Manual agitation at 5-minute intervals in water bath.

<sup>b</sup> End-over-end agitation in an electrically-heated chamber (*This Journal*, 27, 272 (1944)).

<sup>c</sup> Digestion of residue from the single digestion.

of System 1 of Table 1 and in those of the sulfate-containing System 2 of Table 2 were more soluble than the corresponding precipitates formed at 90°C. The opposite effect was obtained, however, for the precipitates from calcium sulfate-containing System 4. It is evident that citrate-solubility of the several precipitates formed in pure systems was not governed solely by P<sub>2</sub>O<sub>5</sub>:CaO ratio.

#### INFLUENCE OF ADDITIVE CALCIUM SULFATE UPON P<sub>2</sub>O<sub>5</sub> TRANSITIONS IN AMMONIATED "TRIPLE" SUPERPHOSPHATE

Experimental concentrated superphosphates were made by phosphoric acid (ortho) acidulations of both raw rock and its defluorinated quenched fusions. After curing one week at 50°C., the acidulates were diluted by additions of calcium sulfate to simulate standard superphosphate and controls were provided by like additions of quartz. It is improbable, how-

ever, that the distribution of the added dihydrate sulfate was as thorough as the dispersal of the anhydrous form generated during the sulfuric acid acidulation of rock phosphate. The experimental mixtures were given full ammoniations in liquid and gaseous parallels and analyzed after 7 and 30 days. The 30-day products were allowed to dry and then were cured 30 days at 50°C. After such curing the products were subjected to aqueous extractions and the water-insoluble residues analyzed for C.I. content.

The effect of the addition of sulfate to the superphosphate from raw rock was apparent in the 7-day development of C.I. in the gaseous ammoniation of Table 4. This effect was increasingly pronounced after 30 days and then was close to that shown for the liquid ammoniation parallel. In the absence of calcium sulfate, there was no great difference between the development of C.I. in the ammoniated mixtures derived from the raw rock and from the fused rock. The calcium sulfate additions that were comparable to the sulfate content of a standard superphosphate proved more retrogressive than the double additions. At the end of the 30- and 60-day periods, available  $P_2O_5$  percentages were comparable in the systems devoid of calcium sulfate and there was no consistent difference in the amounts of C.I. between the aqueous and the gaseous ammoniations. The aqueous ammoniations were, however, much more conducive to the development of C.I. during the aging of the systems that included calcium sulfate.

The "proportion extracted" from the systems containing the sulfate exceeded that from the systems containing the insoluble quartz. In every comparison, however, the citrate-soluble  $P_2O_5$  in the water-insoluble residue was higher for the gaseous ammoniation. The high-solubility of the water-extracted residues from the fused-rock derivative diluted with quartz, and from the raw rock derivative with the double inclusion of quartz, indicates the formation of precipitates other than apatites. The retrogressive effect of the component fluorides is pronounced, in that the mean overall quantity of citrate-soluble  $P_2O_5$  in the systems from brown rock with quartz was only 83 per cent of that found in the systems derived from the fused rock plus quartz.

Possible effect of variance in the dispersal of the additive sulfate was considered in the ammoniations of Table 5, in which the additions were made before and after acidulations. Content of "available" of the acidulates was not affected by variation in the manner of sulfate inclusions. The water-soluble  $P_2O_5$  fraction of every ammoniated product was decreased by the addition of the sulfate. This effect was of variant extent in the 30-day products, but a common level was attained in the 60-day products. The second 30-day period brought increases in the C.I. of all mixtures to which full additions of calcium sulfate were made, but this was not true of the one-half additions made immediately before and after acidulations. Inclusion of the sulfate increased substantially the C.I. content of the

TABLE 4.—Influence of  $\text{CaSO}_4$  upon  $\text{P}_2\text{O}_5$  transitions in superphosphates ammoniated fully with gaseous and with liquid ammonia

			SUPERPHOSPHATES <sup>a</sup>												AMMONIATED SUPERPHOSPHATES													
DERIVATIVE ROCK	ADDITIVE MATERIAL	AMMONIA AS—	P <sub>2</sub> O <sub>5</sub> AS PERCENT OF TOTAL						AFTER 7 DAYS <sup>b</sup>						AFTER 30 DAYS <sup>c</sup>						AFTER 60 DAYS <sup>d</sup>						WATER LEACHING OF THE 60-DAY PRODUCTS	
			P <sub>2</sub> O <sub>5</sub> AS PERCENT OF TOTAL						P <sub>2</sub> O <sub>5</sub> AS PERCENT OF TOTAL						P <sub>2</sub> O <sub>5</sub> AS PERCENT OF TOTAL						P <sub>2</sub> O <sub>5</sub> AS PERCENT OF TOTAL						PROPOR- TION INSOL. RES.	
			AVAIL.	WS.	CS.	CL.	AVAIL.	WS.	CS.	CL.	AVAIL.	WS.	CS.	CL.	AVAIL.	WS.	CS.	CL.	AVAIL.	WS.	CS.	CL.	AVAIL.	WS.	CS.	CL.	C.S.	C.I.
per cent per cent per cent																												
Brown Brown	Quartz	Liquid Gas	98.6	92.0	6.6	1.4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
			98.8	94.1	5.7	0.2	99.3	60.4	38.9	0.6	99.1	67.1	32.0	0.9	98.5	66.7	31.8	1.5	98.5	66.7	31.8	1.5	98.5	66.7	31.8	1.5	30.0	74.1
Brown Brown	CaSO <sub>4</sub> · 2H <sub>2</sub> O	Liquid Gas	96.4	93.6	2.8	3.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
			99.3	90.7	8.6	0.7	92.5	26.5	66.5	3.4	73.4	12.6	60.8	26.6	67.1	11.3	55.8	32.9	46.0	40.4	59.6	46.0	40.4	59.6	44.0	34.4	65.6	
Fused <sup>f</sup> Fused	Quartz	Liquid Gas	99.0	88.7	10.3	1.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
			99.4	89.2	10.2	0.6	94.1	53.8	40.3	1.5	94.3	63.0	31.3	5.7	91.0	66.5	24.5	4.8	91.0	66.5	24.5	4.8	91.0	66.5	24.5	4.8	30.0	94.5
Fused Fused	CaSO <sub>4</sub> · 2H <sub>2</sub> O	Liquid Gas	98.9	93.9	5.0	1.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
			99.3	92.5	6.8	0.7	98.9	37.0	61.9	1.1	88.5	18.4	70.1	11.5	84.1	19.1	65.0	15.9	48.0	55.6	44.4	48.0	55.6	44.4	45.0	33.2	66.8	
Brown Brown	2x Quartz	Liquid Gas	97.9	90.0	7.9	2.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
			99.2	88.8	10.4	0.8	97.9	61.4	36.5	2.1	98.2	65.2	33.0	1.8	98.5	59.1	38.4	1.5	98.5	59.1	38.4	1.5	98.5	59.1	38.4	1.5	20.0	91.7
Brown Brown	{Quartz CaSO <sub>4</sub> · 2H <sub>2</sub> O}	Liquid Gas	96.4	89.1	7.3	3.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
			97.8	87.6	10.2	2.2	95.5	22.5	73.0	4.5	83.7	9.8	73.9	16.3	81.0	10.9	70.1	19.0	32.0	64.3	35.7	32.0	64.3	35.7	30.0	59.2	40.8	
Brown Brown	2x CaSO <sub>4</sub> · 2H <sub>2</sub> O	Liquid Gas	94.8	90.1	4.7	5.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
			97.0	90.5	6.5	2.9	96.1	7.2	88.8	3.9	86.8	1.8	85.0	13.2	85.2	1.8	83.4	14.8	58.0	57.4	42.6	58.0	57.4	42.6	51.0	43.0	57.0	

<sup>a</sup> Cured one week at 50°C.<sup>b</sup> Removed from atmosphere of  $\text{NH}_3$  and placed in sealed containers.<sup>c</sup> The slurries of superphosphates that had stood 30 days at 50°C. were allowed to dry.<sup>d</sup> 30 days after drying of slurries as in (c).<sup>e</sup> From 100 grams of ammoniated products dried at 50°C.<sup>f</sup> The fused rock contained 0.02% of fluorine.





water-insoluble residue from all of the 60-day products other than the one from the superphosphate to which the half portion of sulfate had been added after acidulation.

The effect of the degree of hydration of the additive calcium sulfate and the time of the addition were considered in the ammoniation of superphosphates made by  $H_3PO_4$  acidulations of fused brown rock. Regardless of variation in its degree of hydration, calcium sulfate exerted no effect upon completeness of the  $H_3PO_4$  acidulations, all of which were more complete

TABLE 7.—*Effect of variation in citrate digestions upon solubilities of aged fully ammoniated superphosphates\**

STARTING SOLIDS	NH <sub>3</sub>	P <sub>2</sub> O <sub>5</sub>						
		TOTAL	CITRATE-INSOLUBLE			CITRATE-SOLUBLE		
			PERIODIC AGITATION <sup>b</sup>	CONTINUOUS AGITATION <sup>c</sup>		PERIODIC AGITATION <sup>b</sup>	CONTINUOUS AGITATION <sup>c</sup>	
			SINGLE 1:100	SINGLE 1:100	DOUBLE 1:100	SINGLE 1:100	SINGLE 1:100	DOUBLE <sup>d</sup> 1:100
Raw rock and CaSO <sub>4</sub>	aqua	<i>per cent</i> 38.3	<i>per cent</i> 25.3	<i>per cent</i> 19.0	<i>per cent</i> 9.40	<i>per cent</i> 33.9	<i>per cent</i> 50.4	<i>per cent</i> 75.5
	gaseous	39.4	23.5	21.3	11.30	34.0	45.9	71.3
Fused rock and CaSO <sub>4</sub>	aqua	35.6	23.8	14.8	6.20	33.1	58.4	82.6
	gaseous	36.3	16.1	15.4	4.15	55.6	57.6	88.6
Raw rock and 2CaSO <sub>4</sub>	aqua	32.6	18.6	12.3	2.50	43.0	62.3	92.3
	gaseous	37.6	16.0	15.8	4.40	57.4	58.0	88.3

\* The second, fourth, and seventh systems of Table 4.

<sup>b</sup> Manual agitation at 5-minute intervals in water bath.

<sup>c</sup> End-over-end agitation in an electrically-heated chamber.

<sup>d</sup> Digestion of the residue from the single digestion.

than the sulfuric acid control acidulation. The substantial proportions of water-soluble  $P_2O_5$  attributable to diammonium phosphate in all of the ammoniated products from the  $H_3PO_4$  acidulates is in contrast to the virtual absence of water-soluble  $P_2O_5$  in the ammoniated product derived from the sulfuric acid acidulate.

Three of the residues from leachings of 60-day ammoniated products were evaluated by periodically-agitated and continuously-agitated single and double extractions with ammonium citrate. The results of Table 7 demonstrate that the continuous agitation effected greater dissolvement of all of the six ammoniated products derived from three acidulates of raw and fused rock fortified with additive calcium sulfate. A marked increase in the citrate-soluble  $P_2O_5$  content of every ammoniated solid was obtained by the double extraction. The results demonstrate that the use of a 1-gram charge of material composed chiefly of tertiary phosphates imposes an undue load on the conventional 100 ml. of the neutral ammonium

citrate dissolvent. When the conditions imposed are so extreme as those employed in the making of the present experimentally ammoniated products, there appears to be no distinction between the ultimate effects by aqueous and gaseous ammoniations.

### SUMMARY

Reagent mono- and dicalcium phosphates and experimental superphosphates with variant proportions and forms of calcium sulfate were used in studies as to the immediate and ultimate transitions induced by aqueous and gaseous ammoniations at 30° and 90°C.

The partially C.S. tricalcium phosphate formed in contact with generated solute diammonium phosphate in absence of calcium sulfate at room temperature was rendered less soluble by aging and by elevation of temperature.

Dicalcium phosphate of virtually complete solubility was formed and stabilized in contact with generated solute monoammonium phosphate, with and without incidence of calcium sulfate.

Digestion of suspensions of reagent dicalcium phosphate in contact with solute diammonium phosphate at room temperature induced a relatively insoluble tertiary that became less soluble during aging, whereas the reverse progression occurred in identical systems aged at 90°C. In corresponding systems, the inclusion of calcium sulfate caused disappearance of solute  $\text{PO}_4$  and repressed the solubility of the tertiary precipitate at 90°C.

In the citrate digestion of the reagent-derived tertiaries, continuous agitation gave C.S. values higher than those obtained by periodic agitation, and double digestions registered substantial increases in C.S. values.

Inclusions of calcium sulfate in the experimental concentrated superphosphates resulted in marked decreases in W.S. and substantial increases in C.I. during the curing of the products from both aqueous and gaseous ammoniations. In every comparison of the water-insoluble residues of the cured products, however, the C.I. content was high for the aqueous ammoniation, and the lowest C.I. values were obtained in the absence of component fluorides. The proportion of included sulfate governed the extent of C.I. induced by ammoniation, but there was no effect from variations in manner of introduction or from variance in the form of the sulfate. Continuous agitation during citrate-digestion of the superphosphate-derived tertiaries gave lower C.I. values, as did dual extractions.

The results confirm previous conclusions as to necessity for delimitations in the ammoniation of standard superphosphates and demonstrate that, under temperature control, concentrated superphosphates can be ammoniated highly without serious increase in C.I. percentage. The findings support related evidence that development of hydroxyapatite in am-

moniated superphosphates is conducive to the successive formation of calcium fluorophosphate, or apatite.

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#### MOISTURE CONTENT OF DISTILLERS' BY-PRODUCTS

By WERNER BAUMGARTEN, LEONARD STONE, and C. S. BORUFF  
(Research Department, Hiram Walker & Sons, Inc., Peoria 1, Ill.)

The determination of moisture in many natural products is not an easy task.<sup>1,2</sup> Reproducibility is often questionable. During the determination decomposition may occur, and at times it is hard to detect.

Distillery by-products are becoming more and more important as feed stuffs, and it is therefore desirable to standardize methods for the determination of their moisture content, particularly since close moisture control of the finished materials is essential. Furthermore, incidents have been reported where storage bins containing distillers' dried grains have ignited spontaneously, and excessive or too low moisture content was a contributing factor to the combustion.

<sup>1</sup> W. L. Porter and C. O. Willits, *This Journal*, **27**, 179 (1944).

<sup>2</sup> L. Sair and W. R. Fetzer, *Cereal Chem.*, **19**, 635 (1942).



The method for the determination of moisture used in most control laboratories is drying in an air oven at elevated temperatures. In such a method the sample size, surface exposed, and length of drying time are important factors. In this paper the available methods are compared in order to establish a satisfactory procedure.

The materials investigated, distillers' dried solubles and distillers' dried grains with solubles, are defined by the Association of American Feed Control Officials as the residues obtained in the manufacture of alcohol and distilled liquors after the removal of the alcohol. Distillers' dried solubles are the dried, water-soluble part of the total stillage obtained by removing the coarse particles on screens and by centrifuging, whereas distillers' dried grains with solubles comprise the total dried stillage.<sup>3</sup> Samples were taken from regular carload lots. During the course of the investigation the composition of the raw material ("mash bill") used in the manufacture was changed. In consequence fermentation by-products from corn as well as from wheat were investigated.

The composition of the mash bill is as follows:

		<i>per cent</i>
Mash Bill No. 1 (Samples 1, 2)	Corn.....	86.64
	Rye.....	3.06
	Barley Malt.....	10.30
Mash Bill No. 2 (Samples 3, 4, 5)	Wheat.....	67.50
	Granular Wheat Flour.....	17.89
	Rye.....	3.21
	Barley Malt.....	11.40
Mash Bill No. 3 (Samples 6, 7)	Corn.....	28.13
	Wheat.....	48.58
	Granular Wheat Flour.....	10.56
	Rye.....	2.80
	Barley Malt.....	9.93

The following procedures were examined:

- (1) Drying in air at elevated temperatures.
- (2) Drying in vacuum with and without the use of drying agents.
- (3) The Bidwell-Sterling distillation procedure.<sup>4</sup>

Table 1 shows the moisture content of distillers' dried solubles determined under different drying conditions.

Higher drying temperatures always led to higher results, and decomposition of the samples is shown by darkening of the color after long periods of drying. In vacuum drying the initial loss of weight is great but "loss of weight" curves flatten out after several hours. In the cases where no decomposition was observed it was necessary to continue drying in vacuum

<sup>3</sup> J. C. Bauernfeind, J. C. Garey, W. Baumgarten, L. Stone, and C. S. Boruff, *Ind. Eng. Chem.*, **36**, 76 (1944).

<sup>4</sup> *Methods of Analysis*, A.O.A.C., 1940, 353.

for several hundred hours before drying for an additional 72 hours produced no further change in weight.

The determination of moisture by the Bidwell-Sterling distillation procedure has similar limitations. The use of the higher boiling solvents enhances the rate of distillation of the water, but decomposition of the sample was observed to occur.

In Table 1 the results obtained by the use of different solvents are compared. The light brown color of distillers' dried solubles changed to dark brown or black and a characteristic odor was noted. Decomposition due

TABLE 1.—*Results on moisture in distillers' dried solubles*

OVEN METHODS	TIME OF DRYING	SAMPLES						
		1	2	3	4	5	6	7
	<i>hours</i>	<i>per cent moisture by weight</i>						
Air-oven 105°C. †	2		3.6	2.9	2.7	3.3	2.6	3.0
Air-oven 105°C.*	2			4.2	4.0		3.2	3.4
Vacuum-oven, P <sub>2</sub> O <sub>5</sub> , 25°C. †	300		3.1	2.7	2.0	3.0	1.6	2.3
Vacuum-oven 49°C. †	300			3.1	2.1			
Vacuum-oven, P <sub>2</sub> O <sub>5</sub> , 49°C. †	300			3.1	2.3	3.2	2.1	2.8
Vacuum-oven 61°C. †	300		3.6	3.6				
Vacuum-oven P <sub>2</sub> O <sub>5</sub> , 61°C. †	300		5.7	3.6				
Vacuum-oven, 75°C. †	300		10.0 ‡					
<i>Distillation Methods</i>								
Chloroform	12			2.5	1.7	2.9	1.6	2.3
Carbon tetrachloride	12			2.2	1.6	1.6	1.5	2.2
Benzene	12	1.7	1.7	2.6	1.7	2.5	2.0	2.7
Toluene	12	3.8	4.6	5.4	4.2	5.1	4.0	4.6
Xylene	12	6.4	6.6	7.5	7.7		6.7	8.3

\* Aluminum weighing bottle, 63 mm. diameter. Sample size, 5.0 grams, ground to pass 1 mm. screen.  
† Glass weighing bottle, 35 mm. diameter. Sample size, 5.0 grams. Identical results were obtained by use of aluminum weighing bottles, 63 mm. diameter.

‡ Extensive decomposition was observed.

to protein breakdown, resulting in the formation of water-soluble products, which accumulate in the aqueous distillate and increase the apparent moisture content, is indicated by the analysis of the aqueous distillate for its pH and nitrogen content. In Table 2 these results are compiled according to the solvents used; chloroform, carbon tetrachloride, and benzene enhance breakdown only slightly, whereas toluene and xylene contribute considerably to decomposition. However, the nitrogenous material collected does not account for the total error since the amount is insufficient regardless of the solvent used.

Table 3 compares the moisture content of dried distillers' grains with solubles determined under different drying conditions.

Distillers' dried grains with solubles are more resistant toward decomposition at elevated temperatures than are distillers' dried solubles. No distillers' dried grains are manufactured at the present time, and this product was not investigated. A sample of wet sirup, that is the evaporated soluble stillage containing approximately 35 per cent solids, was distilled

TABLE 2.—*Analysis of aqueous distillate when dried distillers' solubles are distilled with various solvents*

SOLVENT	MG. NITROGEN/100 GRAMS OF SAMPLE	pH
Chloroform	0.14	4.5
Carbon tetrachloride	0.62	4.0
Benzene	0.30	4.0
Toluene	19.0	9.4
Xylene	23.0	8.6

The aqueous phase was evaporated to dryness with concentrated  $H_2SO_4$ , decolorized with a few drops of conc.  $H_2O_2$  and the color developed with Nessler's reagent<sup>a</sup> measured in a Coleman spectrophotometer.

TABLE 3.—*Moisture in distillers' dried grains with solubles*

OVEN METHODS	TIME OF DRYING	SAMPLES						
		1	2	3	4	5	6	7
	hours	per cent moisture by weight						
Air-oven, 135°C.*	2	10.1	13.0	13.7	11.1	13.5		
Air-oven, 105°C.†	2	10.5	11.2	11.2	10.6	11.2		
Vacuum-oven, $P_2O_5$ , 25°C.*	300	8.0	9.4	10.4	10.2	10.4		
Vacuum-oven 49°C.*	300		9.6					
Vacuum-oven, $P_2O_5$ , 49°C.*	300	9.2	10.2	10.5	10.4	10.8		
<i>Distillation Methods</i>								
Chloroform	12		9.2	9.5	11.2	10.5	11.5	
Carbon tetrachloride	12		9.0	9.2	9.5	10.5		
Benzene	12	12.4	8.4	9.0	10.1	10.2	10.2	11.0
Toluene	12	12.9	11.5	10.5	11.1	11.2	11.0	11.7
Xylene	12	14.4					12.5	14.0

\* Glass weighing bottle, 35 mm. diameter. Identical results were obtained by use of aluminum dishes, 63 mm. diameter. Sample size, 5.0 grams, ground to pass 1 mm. screen.

† Aluminum dish, 63 mm. diameter. Sample size 5.0 grams.

with xylene, and the distillate did not show the presence of any decomposition products.

The use of chloroform and carbon tetrachloride as solvents in the Bidwell-Sterling distillation procedure has the following advantages: (1) the low boiling point of these solvents decreases the chances for decomposition, which seems to occur with higher boiling solvents—notably xylene; and (2) the suspension of the sample in these solvents eliminates the char-

<sup>a</sup> Hawk and Bergeim, "Practical Physiological Chemistry," 11th ed., p. 707.

ring and burning resulting from contact with the heated flask, and insures better "moisture-solvent" contact.

The moisture content of distillers' dried grains with solubles is best determined by drying in vacuum at 49°C. over phosphorus pentoxide ( $P_2O_5$ ) (Table 3). The distillation with toluene shows higher apparent moisture content; however, decomposition products are detected in the distillate. Distillation with benzene is not accompanied by any noticeable decomposition. The true moisture content is probably between these two limits. The distillation with benzene gives values which are a few tenths of a per cent below those obtained by vacuum drying at 49°C. over  $P_2O_5$ , but might be preferable for routine analyses because of the length of time required in the vacuum drying method.

In Table 4 the moisture values for distillers' dried solubles obtained by

TABLE 4.—*Comparison of moisture content of distillers' dried solubles obtained by different methods*

SOLVENT	SAMPLE OF MASH BILL NO. 2			SAMPLES OF MASH BILL NO. 3	
	3	4	5	6	7
	<i>per cent moisture by weight</i>				
Chloroform	2.5	1.7	2.9	1.6	2.4
Benzene	2.6	1.7	2.5	2.0	2.7
Vacuum, $P_2O_5$ , 25°C.	2.7	2.0	3.0	1.6	2.3
Vacuum, 49°C.	3.0	2.1			
Vacuum, $P_2O_5$ , 49°C.	3.1	2.3	3.2	2.1	2.8

distillation with chloroform and benzene are compared with those obtained by drying in vacuum. The values for chloroform and vacuum drying with  $P_2O_5$  at 25°C. agree closely. With respect to the first three methods in Table 4 it may be noted that solubles from Mash Bill No. 3 give higher values with benzene than do solubles from Mash Bill No. 2. In view of these facts vacuum drying at 25°C. over  $P_2O_5$  is proposed as the method of standardization for distillers' dried solubles. This procedure is not practical for laboratory control work because of the length of time required for the attainment of constant weight. The method most satisfactory for the laboratory seems to be distillation with chloroform in a Bidwell-Sterling apparatus.

No definite conclusion is reached as to which condition is responsible for the susceptibility of the products to decomposition. The fact that wet sirup can be distilled with xylene without appreciable decomposition, whereas dried solubles show considerable decomposition may indicate that the heat to which the solubles is subjected during drying is responsible.

#### SUMMARY

Distillers' dried solubles show a different moisture content when subjected to drying over  $P_2O_5$  at 25° than at 49°C. Drying at 25°C. is favored

because decomposition at higher temperature may occur. Drying in an air-oven at 105°C. leads to slightly higher results.

Decomposition products were found in the aqueous phase when toluene or xylene was used in the Bidwell-Sterling distillation procedure. The water collected in the distillation showed a higher pH than that collected when benzene or chloroform was used. Nitrogenous material could be detected in the aqueous phase by Nessler's reagent.

The moisture content of distillers' dried grains with solubles is determined by drying in vacuum at 49°C. over  $P_2O_5$  or by distillation with benzene.

### STANDARDIZATION OF ASSAY OF PENICILLIN\*

By ALBERT C. HUNTER and WILLIAM A. RANDALL (Food and Drug Administration, Federal Security Agency, Washington, D. C.)

Methods of assay for determining the antibiotic potency of penicillin become more numerous as attempts are made to perform the complete assay within the space of a few hours and, at the same time, to attain greater precision. Several methods beginning with that of Fleming (2) are now described in the literature. If test organisms other than those now accepted for the assay can be used to advantage and if manifestations of interference with microbiological activity other than the direct measurement of inhibition of growth can be satisfactorily utilized as indices of antibiotic potency, additional reports recommending such procedures will appear. The original procedures described by Fleming (2) included both the "furrow" or "gutter" method and a serial dilution method, but neither provided the means for accurate evaluation of potency which was later taken into consideration in the cylinder plate test first described by Abraham, Chain, Fletcher, Gardner, Heatley, Jennings, and Florey (1). This method, since referred to as the cup-plate test or the ring test, has come into wide use probably to the complete satisfaction of no one but with general recognition of its usefulness as an assay procedure for routine work. The details of this method have been described in the cited article by Abraham *et al.* and in reports by Foster and Woodruff (7) and Schmidt and Moyer (10). They need not be repeated here except to point out that the method merely measures the zones of inhibition produced by diffusion of penicillin contained in small open-end cylinders placed on agar plates seeded with the test organism. Sharing favor with the cup-plate procedure, if not actually outgaining that procedure in favor at the present time, is the turbidimetric method, which evaluates the antibiotic potency of

\* Presented at the 45th General Meeting of the Society of American Bacteriologists, New York City, May 4, 1944.

penicillin through measurement of turbidity as correlated with inhibition of bacterial growth. This procedure, proposed as an alternate method of assay by Fleming (2) and by Abraham *et al.* (1), has been improved and the reading of turbidities enhanced by the use of the photoelectric colorimeter. Details of the method have been discussed by Foster (4), Foster and Woodruff (6), and Foster and Wilker (5).

Additional methods and modifications of the old procedures have been suggested. Sherwood, Falco, and de Beer (11) have proposed the use of filter paper discs in lieu of cylinders in the plate test. Joslyn (8) has described a turbidimetric method which permits an assay in four hours. Rake and Jones (9) have presented a method which utilizes hemolysis in evaluating the antibiotic potency of penicillin. Numerous investigators concerned with the problem of penicillin assay have inquired regarding the relative merits of aerobic sporeformers, staphylococci, and certain other bacteria as test organisms, all with the aim of reducing the error inherent in such a bioassay procedure and perhaps with the hope of arriving at potency values having some meaning in terms of therapeutic dosage.

Fundamentally, of course, the methods proposed measure inhibition of bacterial growth. All the methods, if in any degree quantitative, require the use of a penicillin standard. The need for the development of a standard to permit expression of potency in some terms, no matter how arbitrary, was met by the early investigators in England. Since, at that time, a chemically pure salt of penicillin was not available, the unit of potency which later came to be known as the Oxford unit or the Florey unit came into existence. The standard then used by Abraham *et al.* (1) was a partially purified solution of purely arbitrary strength in dilute phosphate-buffer saturated with ether. It gave an average assay value represented by a zone of 24 mm. diameter on the cup-plate test. The sponsors then adopted as the arbitrary unit that amount of penicillin which when dissolved in one cubic centimeter of water gave the same inhibition as this standard. Later, Florey and Jennings (3), for the benefit of those who used the series dilution method of assay, defined the Oxford unit as that amount of penicillin which when dissolved in 50 cc. of meat extract broth just inhibits completely the growth of the test strain of *Staphylococcus aureus*.

The impracticability of using the diameter of the zone of inhibition as a standard, in all laboratories in this country and under all the conditions encountered, soon became apparent. It was necessary for those who were willing to sponsor a standard for use in the laboratories of the United States to restandardize against the Oxford unit, salts of penicillin which could be distributed as reference standards. These were then used for the preparation of further standards to be used in routine assays. This procedure resulted either in the use of a standard curve based on data ob-

tained in the laboratory distributing the reference standard, or in the preparation of a new standard curve for each testing laboratory based upon assay of the salt of penicillin selected for daily use as referred to a specimen of penicillin which had been assayed against the Oxford standard. Even though the reference standard was obtained from reliable sources of manufacture or through a generous and helpful service rendered by the Northern Regional Research Laboratory of the U. S. Department of Agriculture, difficulties developed as the number of laboratories making assays increased.

In the fall of 1943, the U. S. Food and Drug Administration was called upon to start a regular program of penicillin assays in connection with the enforcement of the Federal Food, Drug, and Cosmetic Act and as a service to other branches of the Government. Penicillin is a "new drug" as that term is defined within the meaning of Section 505 of the Food, Drug, and Cosmetic Act. The law requires that before shipping a new drug in interstate commerce, the manufacturer shall obtain an effective new drug application by filing adequate information to permit appraisal, among other things, of the methods of preparation of the drug and of the methods of control which are applied to guarantee its safety. As a part of the procedure the manufacturer shall also submit to the Food and Drug Administration such samples as may be required by the Administrator of the Federal Security Agency. Arrangements were made and have been followed for the submission to the Food and Drug Administration of samples from each commercial lot of penicillin manufactured, for potency assay and for other appropriate tests bearing upon the question of its safety for use.

The difficulties of the assay became apparent before this program had proceeded more than a few weeks. Inexplicably wide discrepancies were encountered between potency values assigned by the manufacturer and those ascertained by the Food and Drug Administration. The explanation might lie in any one, or combination, of the many variables inherent in the assay procedure, including the lack of a penicillin standard available for universal use. The variations in assay are illustrated by data gleaned from the records covering the examination of penicillin during the initial three months of the program. These data are presented in Table 1.

Taking the products of Manufacturers D and G as examples, assay values for the contents of an ampul were obtained 130 and 101 per cent higher than those declared on the label. On ampuls representing other lots from the same manufacturers assay values 40 and 65 per cent below the declared potency were obtained. It is interesting to note, for comparison with other data to be presented, that the average assay determinations made by the Administration during the period covered by Table 1 were substantially higher than those of the manufacturer.

TABLE 1.—*Variation between manufacturers' assay and F. & D. A. assay*  
October–December 1943

MFG.	SAMPLES	CHECK	ABOVE POTENCY (%)				BELOW POTENCY (%)				MAX per cent	MIN. per cent	AVERAGE per cent
			1-10	11-20	21-30	>30	1-10	11-20	21-30	>30			
A	25	0	6	5	4	3	2	3	1	1	81+	60—	5+
B	6	0	0	3	1	1	0	1	0	0	56+	11—	14+
C	5	0	1	0	1	1	2	0	0	0	160+	7—	43+
D	36	0	4	3	7	12	3	3	2	2	130+	40—	27+
E	17	0	3	5	1	3	3	1	1	0	62+	27—	6+
F	46	1	10	5	5	10	8	3	3	1	95+	49—	12+
G	71	1	9	11	10	14	3	5	5	13	101+	65—	12+
H	8	0	2	2	1	0	2	1	0	0	29+	16—	6+
I	10	0	2	3	1	1	2	0	0	1	58+	40—	9+



As indicated previously, one of the variables that had to be resolved was the reference standard. By common consent, the responsibility and sponsorship for a penicillin standard were placed upon the Food and Drug Administration at least until some other agency is in position to assume the burden.

With the preparation of crystalline penicillin sodium even on a very limited research laboratory scale, it became possible to create a master standard consisting of the chemically pure sodium salt. The present scarcity of the crystalline material prohibits its distribution for use as a standard at this time. However, this situation is taken care of by the use of a secondary or reference standard with a value determined by assay against the master standard.

Through the generosity of three of the larger producers of penicillin, a quantity of pure crystalline penicillin sodium sufficient for the purpose was donated to the Food and Drug Administration for use as the primary standard. The contribution of each manufacturer was studied by evaluating such of its properties as seemed pertinent to determine its identity, and when it was found that the three lots were identical they were mixed and the pooled lot was assayed. At this stage of the standardization program, there were clear indications that the potency of the crystalline sodium penicillin was such that, for purposes of potency description, it would be possible to abandon expressions of potency in terms of the Oxford unit in favor of milligrams of active penicillin without disturbing the clinician or confusing the results of all work heretofore done on the Oxford unit basis. If, as it appeared from the assays, the value of the crystalline material in terms of Oxford units was approximately 1600 per mg., transition to measurement in terms of milligrams could be accomplished without serious disturbance to existing manufacturing and ampul-filling practices.

Eight collaborating laboratories, comprising seven manufacturers of penicillin and the Northern Regional Research Laboratory at Peoria, were asked to assay the pooled lot of crystalline penicillin sodium against the standard currently being used in those laboratories. The requested collaboration was enthusiastically given. The results of this rather extensive series of assays are summarized in Table 2.

From the data in Table 2 it is obvious that an assigned value of 1650 units per mg. for the pure crystalline penicillin sodium would not be materially out of line with its Oxford unit value. Therefore, a potency of 1650 units per mg. has been ascribed to the crystalline penicillin now adopted as a master standard. As a matter of equivalents, this means that the ampul of an impure salt of penicillin designated as containing 100,000 units will contain the antibiotic activity of 60 mg. of pure penicillin sodium and may be so labeled. Simple computation shows, of course, that 60 mg. is really 99,000 units in terms of the master standard, which is sufficiently close for label declarations of potency and obviates the neces-

sity of establishing the value of the standard at a figure carried to the last digit or perhaps to the decimal. The unit although reasonably close is not the Oxford unit. The suggestion is made that the unit as an expression of potency might be carried only until routine practice has come to accept measurement of potency in terms of weight of pure penicillin.

As a reference standard, the Food and Drug Administration has been supplied by one of the large manufacturers with a quantity of penicillin calcium of sufficient size to permit distribution to those who have a legiti-

TABLE 2.—*Collaborative assays of crystalline penicillin sodium for use as primary standard*

LABORATORY	RANGE OF ASSAY OXFORD UNITS/MG.	AVERAGE OXFORD UNITS/MG.	REMARKS
A		1515	28 assays—14 days
C	1617–1914	1749	
D	1290–1710	1480	14 assays
E	1650–1665	1660	Cup-plate assay
	1682–1715	1699	Turbidimetric assay
F	1450–1610	1542	Series dilution assay
	1319–1642	1480	Cup-plate assay
		1580	Turbidimetric assay
H		1596	37 assays
I		1595	2 standards used
NRRL	1350–1615	1494	<i>Staph. aureus</i> (a) standard
	1535–1600	1569	<i>Staph. aureus</i> (b) standard
	1465–1760	1592	<i>B. subtilis</i> (a) standard
	1890–2110	1972	<i>B. subtilis</i> (b) standard
F&DA	1426–1780	1566	9 assays in 2 months
	Average	1606	

mate need for it. The same laboratories where assistance was obtained in arriving at a value for the master standard were asked to assay this calcium salt against the master standard at a value of 1650 units per mg. The values obtained by the nine participating laboratories are presented in Table 3.

Unless or until there is a demonstrated loss of potency of this calcium salt, it is to be used as the reference or secondary standard with an assigned value of 370 units per mg. as related to the master standard at 1650 units per mg. Referred to the primary standard, each milligram of the secondary standard has the equivalent of the activity of 224 micrograms of the pure crystalline penicillin sodium. As long as the Food and Drug Administration bears the responsibility for the maintenance and distribution of the standard, both the master standard and the secondary standard will be assayed periodically to follow closely the stability of the

crystalline salt and to provide assurance as to the relationship of the two standards to each other.

In January 1944, this Laboratory started using crystalline penicillin sodium as the standard, at a value then ascribed as 1600 units per mg. We also utilized the calcium salt secondary standard with a value of 350 units per milligram at that time given to it. As soon as the progress of the standardization program permitted, these values were changed to 1650 for the master standard and 370 for the secondary standard, and assays since then have been made on that basis. The data in Table 1 show the

TABLE 3.—*Collaborative assays of penicillin calcium (against crystalline standard at 1650 u./mg.) for use as secondary standard*

LABORATORY	RANGE OF ASSAY	AVERAGE	REMARKS
A		370	20 assays—10 days
C	344–352	348	
D		376	16 assays
E	378–427	394	Cup-plate assay
F	294–380	343	Series dilution assay
	400–416	408	Cup-plate assay
		362	Turbidimetric assay
H		365	Series dilution assay
I		385	
NRRL	362–414	379	<i>Staph. aureus</i>
	363–406	380	<i>B. subtilis</i>
F&DA	334–373	352	10 assays in 2 months
	Average	372	

wide variations between the manufacturers' assays and Food and Drug Administration assays on ampuls submitted during the initial three months of activity. In Table 4 data similarly compiled from assays made during a period of approximately three months following the adoption of the new standard show better agreement.

With a few exceptions where Government assays were notably out of line with those of the manufacturer, which might be explained by any of several factors, the variations are probably no greater than may be expected in bioassay procedures conducted on entirely different samples of a drug product, even though derived from the same parent lot of material. It is noteworthy that, contrary to the trend of the first three months, the averages of Government assays for each manufacturer's penicillin for the period covered by Table 4 were generally below those of the producer and in those two instances where they were greater, the difference was materially reduced.

The authors are entirely conscious that there are numerous factors other than the standard influencing the assay which need standardization. We have in mind: the test organism to be used, the density or amount of the



inoculum, the composition and amount of the culture medium, the period and temperature of incubation of the test plates or tubes, and the method used for computing the value of the unknown sample against the standard, especially in the plate method. Undoubtedly, there are other factors affecting the assay which should be subject to more precise control than is now possible with our limited knowledge. However, it is doubtful that any thing more than impressions of their relative importance could be presented with the present paucity of information. Certainly there is no intention of going into such details in this report. Eventually, a truly standardized assay procedure will of necessity cover these items. At this time it is sufficient to announce the establishment of the penicillin standard and to express the hope, and possibly the prediction, that the universal adoption of the standard will be reflected in more consistent assay results.

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#### DETERMINATION OF CARBONATES IN FEEDS

By G. S. FRAPS (Agricultural Experiment  
Station, College Station, Texas)

Calcium carbonate is now being added to many mixed feeds, but especially to chicken feeds, for the purpose of supplying calcium, and 5 per cent is also sometimes added to such feeds as cottonseed meal to balance the phosphorus content. Carbonates are naturally present in bone meal and meat by-products containing bone, but occasionally excessive quantities are present. Carbonates in feeds can be determined by modifications of the A.O.A.C. method used for baking products.<sup>1</sup> The modifications involve strength of acid, quantity of sample, and shaking of the mixture of sample and acid, and they also require an evolution flask so connected to

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1940, 186, 4-6.

the apparatus with rubber tubing that the flask can be shaken. The conditions required to give accurate results were studied by J. F. Fudge in connection with various feeds alone and also when mixed with 5 per cent of oyster shell of known calcium carbonate content. With oyster shell alone, low results were obtained when sulfuric acid (1+5) was used, the carbon dioxide evolved being equivalent to only 82.7 per cent of the calcium carbonate known to be present. With 1+9 hydrochloric acid, the calcium carbonate content was 100 per cent of that known to be present. With one sample of bone meal 20 ml. of hydrochloric acid 1+9 gave only 1.38 per cent calcium carbonate, while 20 ml. of 1+2 acid gave 6.38 per cent.

The manner and extent of shaking affected the results. Wheat products

TABLE 1.—*Percentage of CO<sub>2</sub> expressed as CaCO<sub>3</sub> in some feeds*

Alfalfa leaf meal:	.13, 19, .15.
Bone meal:	2.33, 1.94, 4.81, 5.24, 4.63, 7.08, 1.60, 2.03, 2.47, 2.42, 2.52, 2.29.
Buttermilk, dried:	.07
Cottonseed meal:	.03, .03, .05, .03, .05, .11, .05, .03, .10, .10
Dried citrus pulp:	.15
Fish meal:	.68, .45
Gluten feed:	.03, .08
Dried kelp:	.96
Linsced meal:	.15
50% protein meat and bone scraps:	1.19, 1.42, .86, 1.33, 1.45, 1.29, 2.28, 1.54, 1.60, 1.63, 1.21, 1.33
Oats, whole:	.05
Peanut hay:	.10
Rice bran containing precipitated carbonate of lime:	1.98, 2.92, 1.60, 1.64, 1.93, 3.09, 3.20
Rice polishings:	2.57, 1.27, .41
Tankage:	1.15
Wheat bran and screenings:	.10

formed a pasty mass, which held carbon dioxide, and continuous, vigorous shaking for 5 minutes was required to secure correct results. With intermittent shaking for only 2 minutes during a period of 5 minutes, results averaged 0.65 and 0.40 per cent too low for wheat bran and wheat gray shorts, respectively. The percentages of carbon dioxide, expressed as calcium carbonate, found in some samples are given in Table 1. Small amounts of gas were measured from all the samples of unmixed feeds. The quantity was equal to less than 0.15 per cent calcium carbonate with most of the feeds, but with two samples of wheat gray shorts it was 0.31 and 0.36 per cent, respectively. Whether this gas is occluded air or absorbed carbon dioxide, or is due to decomposition of uronic acid compounds has not been ascertained. Carbonates are found in bone meal, meat and bone scraps, tankage, fish meal, rice bran, and rice polish. There would be suspicion of added calcium carbonate if the quantity in bone meal exceeds

2.5 per cent, or if it exceeds 1.5 per cent in meat and bone scraps. Carbonate of lime is used by some rice millers to aid in polishing the grains of rice, and this explains its presence in rice polish and rice bran.

The details of the method used for the determination of carbon dioxide, to be calculated to calcium carbonate, are as follows:

#### METHOD OF ANALYSIS

##### APPARATUS

Use for the decomposition bottle a 6 oz., low, wide-shaped sample bottle fitted with a rubber stopper and rubber tubing. It should be capable of containing a glass vial, 20×70 mm., covered with adhesive tape to minimize breakage. Connect the bottle directly by rubber tubing to a gas-measuring apparatus similar to that used for CO<sub>2</sub> in baking powder<sup>2</sup> and which is filled with the solution prescribed in Section 4 (same reference).

##### DETERMINATION

Place 7 grams or the factor weight\* of the mixed feed in the decomposition flask, put 20 ml. of HCl (1+9) in the vial inside the flask, and connect the flask with the gas-measuring apparatus. Allow the apparatus to stand a few minutes to equalize the temperature. Equalize the pressure by leveling the liquid and opening the stopcock from the bottle to the outside air, then from the gas tube. Lower the leveling bulb to reduce the pressure within the apparatus; then tilt the decomposition flask so the acid comes in contact with the sample, and vigorously agitate the flask 5 minutes to secure intimate mixture of the contents.

If the factor weight is used, the number of ml. of gas evolved divided by 20 gives the CO<sub>2</sub> expressed as percentage of CaCO<sub>3</sub>. Read the temperature and pressure again to see if there is any change. If the CO<sub>2</sub> exceeds 110 ml., repeat, using half the sample weight previously taken.

If the feed mixture gets thick, use continuous and vigorous shaking. For thin mixtures, shake 1 minute vigorously at start and finish.

\* Ascertain the temperature in °C., read close to the CO<sub>2</sub> apparatus. Read the barometric pressure in mm. of mercury, then find the factor weight to be used (calculated by multiplying the weight of 1 liter of CO<sub>2</sub> under like conditions, obtained from the table,<sup>3</sup> by 4.5496). Weigh the sample and do the work immediately as temperature and pressure may change. For bone meal, use 20 ml. of HCl (1+2) and half the factor weight; for meat products containing much bone meal, use 1+2 acid but full factor weight; and when oyster shell or limestone is to be tested use 1/20 of the factor weight.

## DETERMINATION OF ELEMENTAL SULFUR IN INSECTICIDES AND FUNGICIDES

By G. S. FRAPS and T. L. OGIER (Texas Agricultural Experiment Station, College Station, Texas)

Elemental sulfur is used in considerable quantities as an insecticide and fungicide. It is applied as a dust or in a spray, in the latter case being

<sup>2</sup> *Methods of Analysis*, A.O.A.C., 1940, 186.

<sup>3</sup> *Ibid.*, 719.

mixed with chemicals which cause it to be wet by water (wetttable sulfur). It is used alone or mixed with other insecticides or fungicides. When used as a dust, the fineness is often specified, such as "93 per cent passes a 325-mesh sieve." The statement of fineness is required by some state laws.

In spite of the large quantities sold, the A.O.A.C. has no method for the determination of elemental, or free, sulfur in such insecticides and fungicides. The method here suggested for further study is based upon the well-known fact that crystalline sulfur is easily dissolved by carbon disulfide, and on the further fact that other carbon disulfide-soluble substances are usually absent. The sulfur so dissolved is usually oxidized by

TABLE 1.—*Free sulfur in some insecticides and fungicides*

NAMES	CLAIMED per cent	FOUND per cent
Sulfur-calcium arsenate	63.0	64.56
Sulfur-copper arsenate	63.0	64.74
Sulfur-calcium arsenate	61.0	61.23
Sulfur-calcium arsenate	63.0	62.67
Sulfur-calcium arsenate	49.7	48.99
Dusting sulfur	99.5	99.65
Calcium arsenate and sulfur	66.3	65.30
Dusting sulfur	93.0	93.70
Sulfur-calcium arsenate	49.0	50.75
Sulfur-calcium arsenate	70.0	70.87
Dusting sulfur	99.5	99.71
Sulfur-calcium arsenate	46.0	46.20

bromine and determined as barium sulfate,<sup>1</sup> Amorphous sulfur is not soluble in carbon disulfide but appreciable amounts of it are not likely to be present in insecticides such as are here considered. Amorphous sulfur is converted by heat into crystalline sulfur. Amorphous sulfur in sublimed sulfur may also be determined by extracting the crystalline sulfur and weighing the residue.<sup>2</sup>

The quantity of carbon disulfide to be used and the number of washings were decided upon after a number of preliminary tests. The method was tested by washing the samples as described, drying, and weighing the crucible, and then washing again with carbon disulfide. There was no further loss in weight.

#### THE METHOD

Use a frittered-glass filtering crucible or a Gooch crucible prepared with asbestos and dried. Using suction, wash crucible two or three times with CS<sub>2</sub>. Since CS<sub>2</sub> will ignite easily keep it at least 6 feet from a flame. Dry in an oven at 100°C. for 1 hour or longer. Place in a desiccator, cool, and weigh. Weigh 2 grams of the sample into the crucible and dry at 100°C. for not more than 2 hours. (If the crucible is dried longer there may be loss of sulfur.) Cool in desiccator, and weigh. The loss in weight is moisture. Report as water to 0.01 per cent.

<sup>1</sup> *This Journal*, 25, 349 (1942).

<sup>2</sup> Scott, "Standard Methods of Analysis," 4th ed., 519a.



Place the crucible on the filtering flask, add 10 ml. of  $\text{CS}_2$ , and stir gently with a glass stirring rod, breaking up any lumps. Using suction, draw off the  $\text{CS}_2$ , and then wash four times with 10 ml. portions of  $\text{CS}_2$ , using suction. Dry at  $100^\circ\text{C}$ ., cool in a desiccator, and weigh. Subtract this weight from the weight obtained after drying the crucible and contents. Report the difference as free sulfur, calculated to 0.01%.

Some analyses of commercial samples of sulfur-bearing insecticides are given in Table 1. The method seems to be well adapted to checking the guarantee made on sulfur and sulfur mixtures. It is of course not applicable to mixtures containing fats, oils, or other substances besides sulfur that are soluble in carbon disulfide.

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## IMPROVED APPARATUS FOR MOISTURE DETERMINATION DISTILLATION WITH ACETYLENE TETRA- CHLORIDE (TETRACHLOROETHANE)

By ELMER PHILLIPS and J. D. ENAS (El Dorado Oil Works,  
Oakland, Calif.)

In the vegetable oil industry it is desirable to control the moisture content of the material passing through the mechanical presses to ensure a satisfactory extraction of the oil.

Distillation procedures, both by direct and reflux distillation, have been suggested (1-9). Various immiscible solvents both heavier and lighter than water have been used, but the trend is toward the use of the heavier solvents (10-16) for products which do not contain materials which yield excessive moisture from decomposition at the boiling temperature of the solvent.

During the spring of 1937, a system was developed for the rapid determination of moisture and recovery of the solvent. The several types of apparatus which had been suggested for use with tetrachloroethane (B.P. =  $146.3^\circ\text{C}$ .) were not readily adapted to rapid analyses on a large scale. A satisfactory apparatus for this purpose was devised, and it is described in this paper. The apparatus, of course, could be used with other heavy solvents as well.

### DESCRIPTION OF APPARATUS

*Automatic Control of the Units.*—A battery of four distillation units was equipped with an automatic timer that shut off the gas after a suitable interval of time. The timer was hooked up in series with a 110-24 volt transformer and a magnetic gas valve commonly used in conjunction with an electrical thermostat for household heating units. The magnetic valve controls the flow of gas into a pipe provided with sufficient outlets (equipped with petcocks) to supply the several units of the series; an-

other lead into the pipe is equipped with an ordinary gas valve in case any mechanical defects should arise in conjunction with the automatic control.

*Single Unit of the Series.*—The details of the construction and the dimensions, which must be adhered to closely in order to obtain satisfactory results, are shown in Figure 1. A wide-mouthed, 500 ml. Erlenmeyer flask contains the sample plus sufficient tetrachloroethane to eliminate any burning of the sample. The flask is connected to column B by a No. 26 cork stopper, which is cemented to column B by a resistant, synthetic resin.\* Column B is insulated with several layers of asbestos, which is applied wet and trapped with gauze. After drying the outside is painted with sodium silicate to hold the insulation rigid. Column B leads into condenser C, which in turn conducts the condensed liquids into the buret column D, where the aqueous layer is read by ruler R. The buret is calibrated by marking the heights of 1 ml. increments of water on the ruler, and these divisions are later subdivided into tenths. The return tube, E, eliminates draining during the distillation and also reduces the volume of reagent needed by continuous replacement. The apparatus is constructed entirely of Pyrex glass.

An experimental apparatus was made similar to the units described above, with a ground-glass joint on a petcock attached at hole H. The petcock could be turned either to a vacuum system during distillation or to the atmosphere in order to break the vacuum after a run. A heavy Pyrex glass vacuum flask was substituted for the Erlenmeyer flask. It was thought that by distilling under vacuum more nearly accurate results could be obtained by reducing the boiling points of the liquids. In this way it was hoped that decomposition of sugars and other materials that give water of decomposition could be entirely eliminated. However, entrainment of water occurred in return tube E, which gave unreliable results even when the heat applied was cut to a minimum. It is possible that where greater accuracy is desired some slight alteration in construction and choice of reagent might give more nearly accurate results with vacuum distillation.

Figure 2 illustrates in detail the laboratory stand (U. S. Patent No. 2202265, May 28, 1940).

#### PROCEDURE

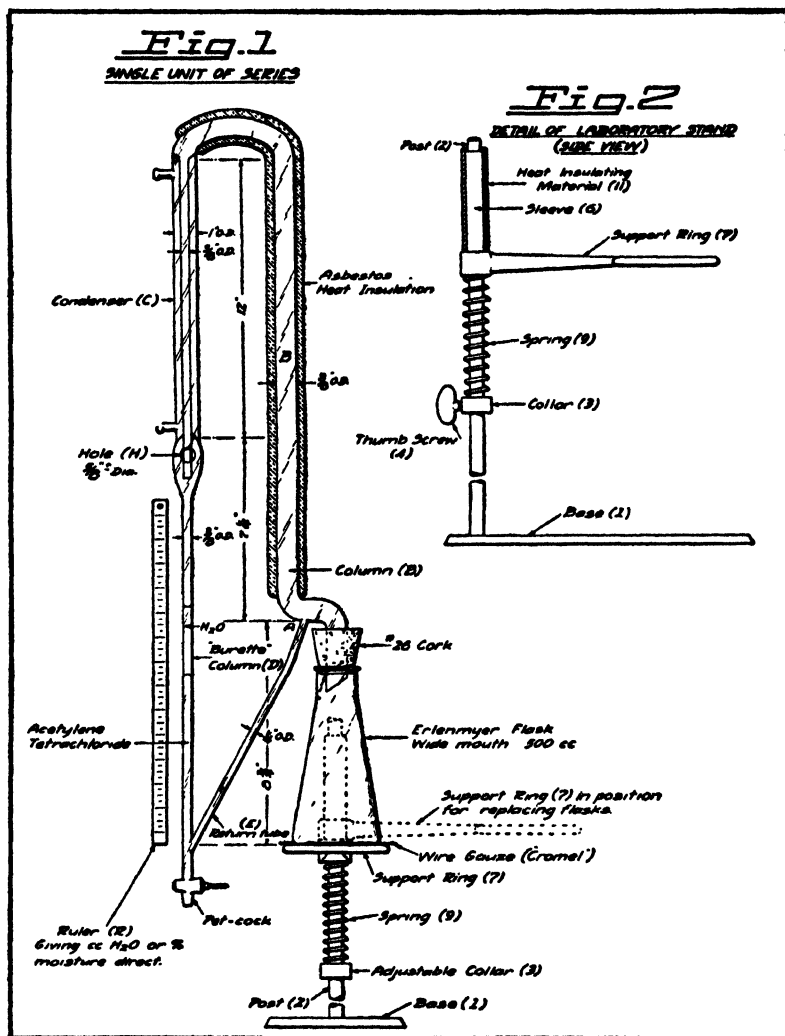
The finely ground material (50 grams) is weighed out into a beaker and transferred into the 500 ml. Erlenmeyer flask by means of a copper funnel constructed to fit snugly inside the neck of the flask; and 150 ml. of tetrachloroethane is introduced into the flask by means of a dispensing buret which is automatically refilled by a slow running siphon. The contents are shaken, and the flask is seated on the cork. While holding it in this position with one hand, support ring 7, which is in "position

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\* A satisfactory resin is sold by the Triangle Paint Co., Berkeley, Calif., under the trade name of "In-sol."

for replacing flasks," (Fig. 1) is depressed and rotated to a position directly under the flask by grasping sleeve 6 (Fig. 2) with the other hand. The spring must be adjusted previously to a suitable tension to insure no possibility of a leak at the junction of the cork and flask. No readjustments are needed for months.

The buret column, D, is filled to the level of point A through the hole H with tetrachloroethane by means of a wash bottle equipped with a pressure bulb. The



automatic timer is turned to 15 minutes, and the gas is lighted. (For consistent results this time should be adhered to closely.) After the elapsed time the operator reads the volume of water with the aid of ruler R. Occasionally it is necessary to loosen some of the tetrachloroethane from the upper meniscus (due to surface tension) by inserting a fine wire through the hole H. Several determinations are run

without draining the buret column D, the number possible depending upon the moisture content of the material being tested.

*Recovery of "spent" tetrachloroethane.*—Probably the most efficient recovery is obtained by steam distillation of the residue (including the sample). Tin appears to be reasonably resistant to the action of this reagent. Because of these facts it was found expedient to construct a steam still with a heavy tin plating and a condensing coil of block tin. The condensed liquids are conducted into a 5 gallon bottle, and the water from the distillation overflows into a sink while the tetrachloroethane remains in the bottle as the heavier liquid phase. The still is emptied by means of a valve and drain leading directly into the sewer system.

The results obtained by this method are usually reproducible within the ability to read the buret column. In comparison with the electric oven, the results for the material used are approximately equivalent to drying for 3.5 hours at 110° C. and consistently higher than those obtained with a 2-hour drying period.

#### PRECAUTIONS

(1) The dimensions should be adhered to closely, as they have been carefully worked out in detail from experience with several experimental pieces of apparatus. Where the return tube E enters column B at A, tube E is shrunk sufficiently to prevent water from adhering to glass A. If a drop of water should cling at A owing to improper proportion of tube E, this remains constant in volume and can be corrected for in the first distillation of a series. The succeeding runs, before draining, have been found to be constant in such a case.

(2) Sufficient heat must be supplied to the flask in order to make this method rapid.

(3) The glass tubing used for the buret column must be of a uniform bore.

(4) After draining, the buret column must be filled to the level of A or water collecting in return tube E will expand suddenly when forced out into column B and may cause a loss of moisture through hole H.

(5) The series should be set up in a fume hood provided with a powerful fan as tetrachloroethane is toxic. (The inside of the fan needs a protective paint.) The wash bottle used to fill the buret column should be provided with a pressure bulb.

#### ADVANTAGES

(1) Ease of manipulation: saves time by use of the automatic timer and the improved laboratory stand.

(2) The ruler R eliminates one subtraction from each determination in a series and is easier to read than the etchings on an ordinary buret.

(3) The danger of leaks due to improper seating between the cork and flask is eliminated by the spring on the laboratory stand.

(4) The recovery system for the solvent is very efficient and convenient.

## ACKNOWLEDGMENTS

The work was suggested by E. A. Seidenspinner, El Dorado Oil Works, San Francisco. The writers are also greatly indebted to W. J. Cummings, College of Chemistry, University of California, Berkeley, for helpful suggestions in the construction of the glass units.

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## IDENTIFICATION OF MONOCHLORACETIC ACID AS BARIUM MONOCHLORACETATE

By JOHN B. WILSON and GEORGE L. KEENAN (Food and Drug Administration, Federal Security Agency, Washington, D. C.)

When considerable proportions of monochloroacetic acid are present and when other acid constituents soluble in ether are not present in excessive quantities, it has been found possible to identify the acid in the form of its barium salt. The procedure used follows:

### PREPARATION OF THE BARIUM MONOCHLORACETATE

To 100 ml. of solution containing 50 mg. or more monochloroacetic acid, add 3 ml. of  $\text{H}_2\text{SO}_4$  and extract with an equal volume of ether in a separatory funnel. In cases where emulsions form, extract in an automatic extractor for 1 or 2 hours. Transfer the ether extract to a separatory funnel and add a few drops of phenolphthalein indicator. Now add 5 ml. of  $\pm 0.1 \text{ N Ba(OH)}_2$  and shake for 30 seconds. If the water layer takes on the pink color of phenolphthalein, transfer through a filter paper to a small beaker. Add  $\pm 0.05 \text{ N}$  acetic acid until colorless and evapo-

rate to 1-2 ml. on the steam bath. Allow the remaining liquid to evaporate spontaneously in the air and finally in a desiccator. If 5 ml. of  $\text{Ba}(\text{OH})_2$  does not give a pink water layer, add 5 ml. more and shake before separating. Repeat the extraction with  $\text{Ba}(\text{OH})_2$  several times or until a pink solution is obtained, evaporating each barium solution in a separate beaker. Examine the crystals under the polarizing microscope.

#### OPTICAL-CRYSTALLOGRAPHIC PROPERTIES OF BARIUM MONOCHLORACETATE

Barium monochloracetate monohydrate crystallizes from water in plates, many of which are hexagonal in habit (Fig. 1), and frequently they form in overlapping layers (Fig. 2). Even in material that has been finely powdered for microscopic examination, the pointed terminations of the

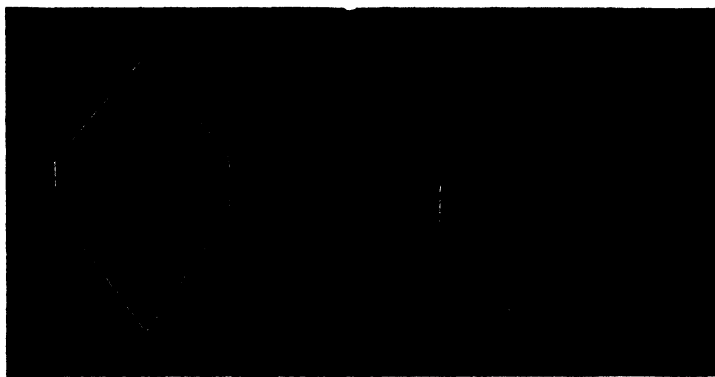


FIG. 1.—BARIUM MONOCHLORACETATE, TYPICAL HABIT

plates, often in pairs, can be observed. In parallel polarized light (crossed nicols), the extinction is parallel and the sign of elongation negative on the more elongated plates. The plates invariably extinguish sharply with crossed nicols and therefore interference figures were not observed in convergent polarized light (crossed nicols). In view of the fact that the plates persistently lie in one orientation the significant refractive indices were determined by the statistical method, and the lowest and highest indices, respectively, were measured on plates showing the maximum amount of double refraction. These two indices are therefore arbitrarily designated as  $n_\alpha$  (the minimum value) and  $n_\gamma$  (the maximum value). The two significant refractive indices are:  $n_\alpha = 1.582$  and  $n_\gamma = 1.611$ , both  $\pm 0.002$ , and frequently shown on the platy fragments.

When applied to water solutions the procedure was successfully used to identify monochloracetic acid when 10 mg. was present in 100 ml. of solution. In the case of creme soda and orange soda packed for the study of the persistence of monochloracetic acid in carbonated beverages<sup>1</sup> the sam-

<sup>1</sup> *This Journal*, 25, 145 (1942).

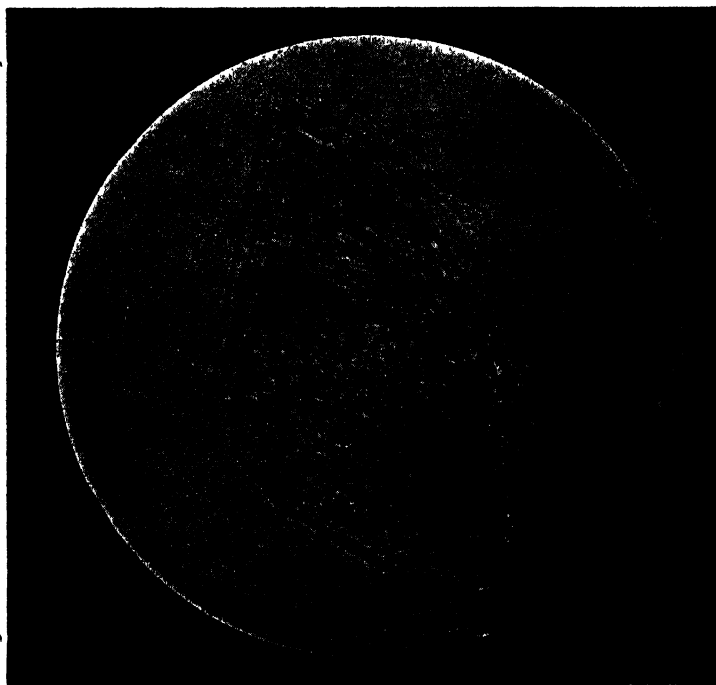


FIG. 2.—BARIUM MONOCHLORACETATE (100×).

ples containing 30 mg. of monochloracetic acid in 100 ml. gave positive tests. From other samples containing substances that interfered with crystallization, no identifiable crystals were obtained.

Solutions containing dichloracetic acid yield a barium compound that produces a vitreous residue, which is hygroscopic. A crystalline barium trichloracetate is formed under the conditions set forth, but so far has not been found suitable for microscopic identification although it crystallizes in a habit distinctly different from barium monochloracetate.

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## HEXABROMIDE METHOD FOR DETECTION OF SMALL QUANTITIES OF LINOLENIC ACID IN ANIMAL FATS

### DETECTION OF HORSE MEAT IN ADMIXTURES WITH PORK OR BEEF

By G. K. CROWELL (Division of Chemistry and Sanitation, New Hampshire State Department of Health, Concord, N. H.)

Hexabromide values have been used by many investigators in oil assay. Bailey and Johnson<sup>1</sup> determined the hexabromide values of salmon oils as

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<sup>1</sup> *Ind. Eng. Chem.*, 10, 999-1001 (1918).

a means of identifying the species of salmon from which the oils were derived. Bailey and Baldsiefen<sup>2</sup> utilized hexabromide values to detect oils other than linseed in paints. Steele and Washburn<sup>3</sup> give in detail a hexabromide method for linseed oil. Paschke<sup>4</sup> observed that fatty acids derived from horse fat gave higher hexabromide values than did those derived from pork, beef, or mutton. Shepard, Fisher, and Bailey<sup>5</sup> report higher hexabromide values than those obtained by Paschke when using the same procedure.

The author, when investigating the Paschke procedure, obtained hexabromide values for horse, pork, and beef fat higher than those reported by Paschke but lower than those reported by Shepard, Fisher, and Bailey. Owing to the wide variance of results obtained with the Paschke procedure a modified method is presented. This method takes into consideration the solubility of the precipitated hexabromide derivative and prevents the precipitation of fatty acids other than hexabromide fatty acids during the bromination procedure. This was found to be extremely important in that it was impossible to obtain duplicate checks unless these factors were controlled.

The fat used for the analyses was extracted from lean, untreated meat which contained an average amount of fatty tissue. The use of straight fat was avoided owing to the known varying composition, depending upon the portion of the animal from which the fat was removed. The meat represents cuts on retail sale at local markets used in the preparation of hamburger steak and sausage. The horse meat was on sale as either steaks or as dog food. All fat mixtures were made from extracted fat and not from the original meat.

The details of the proposed method follow:

#### METHOD

##### EXTRACTION OF FAT

Pass the meat sample twice through a meat grinder, place in a beaker, and warm to about 60°C. in a drying oven. Remove the beaker from the oven and add sufficient petroleum ether to completely cover the meat. After the ether has ceased to boil, cover the meat with more ether and allow to stand overnight at room temperature. Filter the fat-ether mixture through a dry filter and allow the ether to evaporate on a steam bath in the presence of a stream of nitrogen or carbon dioxide. Store the ether-free fat in a refrigerator until used.

##### SAPONIFICATION OF FAT

Reflux 10 grams of the extracted fat with 100 ml. of 0.5 N alcoholic KOH for 30 minutes. Transfer the hot liquid to a distilling flask and remove approximately 80 ml. of the alcohol by distillation. Add 250 ml. of distilled water to the flask and

<sup>2</sup> Ind. Eng. Chem., 12, 1189-1194 (1920).

<sup>3</sup> Ibid., 52-59.

<sup>4</sup> Z. Untersuch. Lebensm., 76, 476 (1938).

<sup>5</sup> Conn. Agr. Exp. Sta. Bull. 475 (1943).



transfer the solution to a large separatory funnel. While the liquid is still warm, add 15 ml. of 5 *N* H<sub>2</sub>SO<sub>4</sub>, 250 ml. of saturated salt solution, and 50 ml. of dry ethyl ether. Shake the funnel vigorously and allow the layers to separate. Withdraw the ether layer and wash with 3–15 ml. portions of saturated salt solution. Filter the washed ether through a dry filter into a 50 ml. volumetric flask and cool to refrigerator temperature (5°–10°C.). Make to volume with dry ether at this same temperature.

#### PRECIPITATION OF HEXABROMIDE

Place 10 ml. (2 grams) of the ether extract containing the fatty acids into a tared centrifuge tube (6"×1"). Add 15 ml. of anhydrous hexabrominated-saturated ethyl ether plus 2 ml. of glacial acetic acid. (The anhydrous ethyl ether was saturated at refrigerator temperature with hexabromide formed from the bromination of fatty acids present in horse fat. A method for preparing hexabrominated saturated ethyl ether is described by Bailey and Baldisiefen.<sup>1</sup>) Cool the tube for 15 minutes in an ice-salt bath, the temperature of which is –5°C. to –10°C. To this cooled solution add from a buret, dropwise, 2 ml. of the brominating solution (2 ml. of C.P. bromine plus 8 ml. of glacial acetic acid). Keep the tube in the ice-salt bath during the bromination and for 15 minutes after. Stopper the tube and place in a refrigerator held at 5°–10°C. overnight. Remove the tube from the refrigerator and place it in an ice bath for 15 minutes. Remove stopper and centrifuge the tube for 2–4 minutes at a speed of 900–1000 r.p.m. (12" head). Decant all the ether from the tube. (There should be no precipitate carried away with the decanted ether provided the centrifuge is run at the suggested speed.) To the tube add 10 ml. of previously cooled anhydrous hexabrominated-saturated ethyl ether (this wash ether is prepared by adding 2 ml. of glacial acetic acid to 25 ml. of the anhydrous hexabrominated saturated ether). Stir the precipitate vigorously and cool the tube in an ice bath for 15 minutes. Centrifuge the tube for 2–4 minutes and decant the ether. (If this procedure is carefully followed, the precipitate remaining in the tube after the last washing should be white.) Should the precipitate still hold some occluded bromine, repeat washings until all color of bromine has disappeared. Avoid excessive washing. Dry the tube at 105°–110°C. for 30 minutes. Record weighed hexabromide in mg./gram of fat.

#### DISCUSSION

The method presented has proved to be more reliable than the Paschke procedure, for the reason that during the cooling procedure no fatty acids are precipitated, and thus any possible error due to incomplete removal, by washing, of non-brominated fatty acids is avoided. The use of anhydrous hexabrominated-saturated ethyl ether in brominating and washing samples is necessary owing to the solubility of the hexabrominated derivative.

The melting point of the hexabromide formed from the fatty acids present in horse fat is in accordance with the literature for linolenic acid hexabromide. Table 1 shows that an increased hexabromide value above that found for pork or beef, together with an identification (melting point) of the prepared hexabromide derivative, would constitute sufficient evidence that horse fat/meat was present.

The method presented obviously does not lend itself to a quantitative estimation of the proportion of horse meat in mixtures with pork or beef,

TABLE 1.—*Results of analysis*

ORIGIN OF FATTY ACIDS	R <sup>40</sup> C.†	IODINE NUMBER (HANUS)	HEXABROMIDE VALUES* (MG./G.) FAT	M.P. HEXABROMIDE	LINOLENIC ACID
Beef	46.6	42.0	2.0	darkens 182°C.	.04
Pork	48.6	59.4	7.5	darkens 182°C.	.14
Horse	53.2	84.8	56.8	179.5°–180°C. (corr.)	1.04
Mixtures:					
beef (%)    horse (%)					
25    +    75			44.0		
50    +    50			28.8		
75    +    25			16.0		
pork (%)					
25    +    75			45.0		
50    +    50			32.5		
75    +    25			19.6		

\* Represent the average of 6 different samples, run in duplicates, none of which varied more than  $\pm 10\%$  from the above recorded values.

† Butyro refractometer reading.

since meat from horses being slaughtered at the present time contains less fat than is usually present in either pork or beef.<sup>6</sup> Products such as hamburger and sausage require considerable fat to be palatable, and it would be generally assumed that this additional fat would be obtained from either the pork or beef and not from the adulterant, horse meat.

## DETERMINATION OF CHOLESTEROL AS BASIS FOR ESTIMATING EGG SOLIDS CONTENT OF NOODLES

By R. C. KOEHN and F. A. COLLATZ (Products Control Department, General Mills, Inc., Minneapolis, Minn.)

In 1941 the cholesterol method<sup>1</sup> for the estimation of the egg solids content of noodles and like products was adopted as official in place of the lipid phosphorus method.<sup>2</sup> The use of cholesterol as an analytical factor for estimating egg solids content is based on the assumption that the percentage of cholesterol is widely different in eggs and in the cereal ingredients of noodles, but is essentially constant among various lots of either type of material. The cholesterol method is distinctly superior to the older lipid phosphorus procedure in that it requires no correction for loss in the manufacturing process or during long periods of storage. In the application of this method the product is saponified, the unsaponifiable portion is treated with bromine to form cholesterol dibromide, and the insoluble dibromide is separated from the reaction mixture by filtration. The dibromide is then taken up in ether and alcohol and hydrolyzed with

<sup>6</sup> Personnel communication—Meat Inspection Division, Livestock and Meats Branch, War Food Administration.

<sup>1</sup> *This Journal*, 25, 63, 93 (1942).

<sup>2</sup> Raymond Hertwig, *This Journal*, 7, 91 (1923).

alkali. The resultant aqueous solution of potassium bromide is oxidized to bromate with hypochlorite and the bromate is determined iodometrically. In the older lipid phosphorus method the lipoids were extracted

TABLE 1.—*Cholesterol content of commercial frozen egg yolks*

SAMPLE	TOTAL SOLIDS	UNSATONIFIABLE MATTER (DRY BASIS)	STEROL AS CHOLESTEROL (DRY BASIS)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	Present Study		
1	45	4.07	2.90
2	45	3.67	2.92
3	45	3.90	2.84
Average	45	3.88	2.87
	Haenni (1941)		
Max.	46.11	4.00	3.15
Min.	40.43	3.39	2.66
Av.*	44.18	3.61	2.88

\* Fifteen samples.

TABLE 2.—*Apparent cholesterol content of durum flour*

SAMPLE	TOTAL SOLIDS	UNSATONIFIABLE MATTER (DRY BASIS)	STEROL AS CHOLESTEROL (DRY BASIS)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	Present Study		
1	88.4	.14	.028
2	86.5	.12	.028
Average	87.45	.13	.028
	Haenni (1941)		
Max.	89.96	.14	.044*
Min.	86.83	.10	.021
Av.†	88.54	.11	.024

\* Next highest value .031%.

† 12 samples durum flour, 6 of semolina, and 6 of patent.

from the noodles with alcohol and ether, and the phosphorus content was determined in the usual manner.

Two lots of durum patent flour and three lots of commercially frozen egg yolks were analyzed by Haenni's<sup>3</sup> revised method for unsaponifiable matter and sterols. In Tables 1 and 2 the results obtained in this laboratory are compared with those obtained by Haenni<sup>3</sup> in 1941.

<sup>3</sup> *This Journal*, 24, 119 (1941).

As is apparent from the data of Table 1, the unsaponifiable matter of egg yolks was found to be somewhat higher than the average previously reported by Haenni. Despite this difference the sterol content of the egg yolks was the same. It therefore appears that a value of 2.88 may be taken as an accurate estimate of the cholesterol content of commercial frozen egg yolks.

In the case of durum flours the values found in this laboratory differ more widely from those previously reported. Both of the two durum flours analyzed were found to contain .028 per cent "cholesterol," whereas the previous study had indicated an average value of .024 per cent. This difference may be due to several variables, such as variety, geographic region milling practice, and seasonal variation in durum flour characteristics. It may be of value to carry out further studies to determine the magnitude, if any, of these variables, and in such a study it would probably be advisable to use the same eggs throughout the study.

To check the validity of the factors given in Tables 1 and 2, three samples of noodles of known composition were analyzed, with the results given in Table 3. Also included in this table are values obtained by the lipid phosphorus method. The data shown in Table 3 are averages of duplicate determinations, all pairs of which agreed closely.

TABLE 3.—*Analysis of noodles of known composition (dry basis)*

SAMPLE	EGG SOLIDS CONTENT	UNSAPONIFIABLE MATTER	STEROL (AS CHOLESTEROL)	CALCULATED EGG SOLIDS		
				1*	2	3
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	5.53	.33	.187	5.7	5.6	5.4
2	5.01	.31	.172	5.2	5.0	4.8
3	7.00	.36	.227	7.1	7.0	6.9

\* 1, Assumes .024% "cholesterol" in durum flour. 2, Assumes .028% "cholesterol" in durum flour. 3, Calculated from lipid P<sub>2</sub>O<sub>5</sub>.

Since the factor .028 per cent "cholesterol" for durum flour was determined on the product actually used in these noodles, it is not surprising that the calculated values for egg solids shown under Column 2 of Table 3 are more accurate than those obtained by using the value .024 per cent and shown under Column 1. On the whole, there is satisfactory agreement between values calculated from "cholesterol" and those actually used in the formula.

The egg solids content determined by lipid phosphorus analyses is consistently low in all three samples analyzed, which fact substantiates the belief that the cholesterol method is more satisfactory for the determination of egg solids content than is the lipid phosphorus technic.

To determine the effect of storage on the apparent egg solids content of noodles, analyses were made of four samples before and after 5-6 months'

storage in paper cartons similar to those used in packing noodles. The data of Table 4 indicate a decrease in lipid phosphorus after 5-6 months' storage, whereas the cholesterol content remained constant. This work confirms Haenni's findings.

In order to provide more information on the relative merits of the cholesterol and lipid phosphorus methods, the authors analyzed five samples of commercial noodles which had been made to contain 5.6 per cent egg solids on a dry basis. The results are shown in Table 5.

TABLE 4.—*Effect of storage on apparent egg solids content of noodles*

SAMPLE	FRESH SAMPLE EGG SOLIDS		5-6 MONTHS' STORAGE EGG SOLIDS	
	FROM CHOLESTEROL	FROM LIPOID P <sub>2</sub> O <sub>5</sub>	FROM CHOLESTEROL	FROM LIPOID P <sub>2</sub> O <sub>5</sub>
	per cent	per cent	per cent	per cent
1		5.5-5.9*	5.6	5.3
2	5.6	5.4	5.6	5.3
3	5.0	4.8	5.0	4.6
4	7.0	6.9	7.1	6.8

\* Composite of noodle samples containing 5.5-5.9% egg solids as determined by the lipid phosphorus method.

TABLE 5.—*Analysis of commercial noodles (dry basis)*

SAMPLE	UNSAPOНИFIABLE MATTER	STEROL (AS CHOLESTEROL)	EGG SOLIDS		
			1*	2	3
	per cent	per cent	per cent	per cent	per cent
1	.31	.186	5.7	5.5	5.4
2	.31	.190	5.8	5.6	5.6
3	.30	.188	5.7	5.6	5.5
4	.30	.192	5.9	5.8	5.6
5	.32	.185	5.6	5.5	5.3
Average	.31	.188	5.7	5.6	5.40

\* 1, Assumes .024% "cholesterol" in durum flour. 2, Assumes .028% "cholesterol" in durum flour. 3, Calculated from lipid P<sub>2</sub>O<sub>5</sub>.

The findings reported in Table 5 confirm those obtained with samples of known composition. The use of the factor .028 per cent gives results which are in excellent agreement with the formula value for egg solids content. When the factor .024 per cent is employed the values tend to run higher, and when the determination is made by the lipid phosphorus method the results are definitely lower.

#### SUMMARY

The cholesterol method for the determination of egg solids of noodles gives excellent results in samples of known composition.

The cholesterol content of durum flours apparently is subject to some variation, which may be due to one or several variables such as variety of grain, growing region, milling operations, and seasonal variations; future studies may be of value to establish the magnitude of these fluctuations.

The lipid phosphorus method gives lower egg solids values than does the cholesterol procedure. Analysis of noodles of known composition shows that the cholesterol method gives the more accurate results.

The apparent egg solids content of noodles as determined by the lipid phosphorus method decreases on storage, whereas the same values calculated from cholesterol content remain essentially constant.

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## DETECTION OF ADULTERATION OF OLIVE OIL USED IN PACKING MAINE SARDINES

By MENNO D. VOTH (Food and Drug Administration, Federal Security Agency, Boston, Mass.)

The scarcity of olive oil and the price differential between it and other edible oils caused by war conditions make the adulteration of the olive oil used in packing domestic sardines exceedingly profitable. The detection of this adulteration demands a knowledge of the effect of the natural fish oils on the chemical constants of the added oil.

In the course of an investigation of the industry it was noted that the Hanus iodine number of the surplus oil drained from the canned fish was not materially different from that of the oil used as a packing medium. It had previously been assumed that the oil from the fish, having a high iodine value, would materially affect the constants of the added oil, during and after processing. In order to test the consistency of the above observations experimental packs were made with sardines packed in various oils.

Small size Maine sardines were prepared in the usual commercial way. Five kinds of oil were used and 20 ml. of each kind was added to each of 10 cans of fish before sealing and processing. Two months later 6 of these cans were opened, the oil drained therefrom, and its iodine number and refractive index determined. Some of the same lot of Maine sardines were smoked in the usual way before packing, and a similar series of experimental packs was prepared. From the results obtained it is evident that when plain or smoked Maine sardines are packed in oil, very little change takes place in the Hanus iodine number or in the refractive index of the packing oil during and after processing because of its blending with the oil in the fish. Tables 1 and 2 illustrate the point. As is usually the case, good correlation was found between the refractive index and the Hanus iodine number. The refractive index can therefore be used as a rapid sorting method.

A limited amount of additional experimental work was performed to

TABLE 1.—*Hanus iodine numbers and refractive indices of drained oil from Maine sardines*

SUB NO.	OLIVE	PEANUT	CORN	COTTONSEED	SOYA
	<i>Hanus iodine number</i>				
1	85.5	96.8	125.0	111.4	130.1
2	85.9	96.3	124.0	111.3	129.0
3	86.0	99.3	124.8	111.9	129.3
4	87.5	97.5	125.5	113.2	130.4
5	86.7	96.5	124.8	112.5	129.7
6	85.8	98.2	125.3	112.1	130.1
Av. iodine No. of drained oil	86.2	97.4	124.9	112.1	129.8
Iodine No. of oil used in canning	83.9	96.9	125.0	112.5	130.1
	<i>Refractive indices at 25°C.</i>				
1	1.4680	1.4698	1.4737	1.4717	1.4735
2	1.4682	1.4697	1.4734	1.4715	1.4737
3	1.4680	1.4700	1.4735	1.4717	1.4735
4	1.4683	1.4698	1.4735	1.4716	1.4737
5	1.4681	1.4696	1.4734	1.4715	1.4736
6	1.4680	1.4697	1.4732	1.4715	1.4737
Av. Ref. indices of drained oil	1.4681	1.4698	1.4735	1.4716	1.4736
Ref. indices of oil used in canning	1.4676	1.4697	1.4735	1.4716	1.4739

TABLE 2.—*Hanus iodine numbers and refractive indices of drained oil from smoked Maine sardines*

SUB NO.	OLIVE	PEANUT	CORN	COTTONSEED	SOYA
	<i>Hanus number</i>				
1	87.1	95.9	125.4	112.6	129.8
2	88.1	98.7	125.2	113.5	129.6
3	89.4	97.5	126.1	111.8	129.7
4	87.7	96.5	124.6	110.8	128.6
5	87.8	95.8	124.7	111.9	129.3
Av. iodine No. of drained oil	88.0	96.9	125.2	112.1	129.4
Iodine No. of oil used in canning	83.9	96.9	125.0	112.5	130.1
	<i>Refractive Indices at 25°C.</i>				
1	1.4682	1.4701	1.4736	1.4718	1.4739
2	1.4685	1.4696	1.4737	1.4719	1.4738
3	1.4685	1.4697	1.4736	1.4717	1.4737
4	1.4686	1.4697	1.4736	1.4717	1.4740
5	1.4684	1.4696	1.4735	1.4717	1.4740
Av. Ref. indices of drained oil	1.4684	1.4697	1.4736	1.4718	1.4739
Ref. indices of oil used in canning	1.4676	1.4697	1.4735	1.4716	1.4739

ascertain whether other varieties of fish which are sometimes canned in oil would show similar results. In order to determine the approximate oil and moisture contents, two kinds of raw fish and four kinds of smoked were comminuted and dried at 100°C.; the oil was extracted with petroleum benzine, and the solvent was evaporated at 70°C. in vacuo. Some of these same kinds of fish were also canned in olive oil and later opened, the oil drained therefrom, and its iodine number determined. Table 3 indicates that the amount of oil in the fish has a direct bearing on the extent of the change that takes place in the iodine number of the drained oil. Only in very oily fish such as smoked mackerel was there a considerable change in the iodine number.

TABLE 3.—*Effect of amount of oil in fish on iodine number*

FISH USED	RAW OR SMOKED FISH		CANNED FISH	
	MOISTURE	OIL ("AS IS" BASIS)	IODINE NO. OF ADDED OLIVE OIL	AV. IODINE NO. OF DRAINED OIL FROM 4 CANS
	<i>per cent</i>	<i>per cent</i>		
Maine sardines (small ocean herring)	76.0	2.2	83.9	86.2*
Large ocean herring	59.7	11.1	—	—
Kippers (lightly smoked large ocean herring)	55.4	12.1	85.5	90.5
Bloaters (heavily smoked large ocean herring)	39.2	15.5	85.5	94.8
Smoked mackerel	46.3	26.6	85.0	99.5
Smoked salmon	—	4.0	85.0	86.5

\* Av. figures from Table 1 (6 cans).

In order to ascertain whether the heat of processing had any effect on the iodine number of oils, the five kinds of oil listed in Table 1, the iodine numbers of which were known, were sealed in sardine cans and processed in the usual way. No change took place in the iodine numbers of any of the oils.

Formerly the method of testing canned sardines for adulterated oil was based on a comparison between the iodine number of the drained oil as actually determined and as calculated from the known iodine number of the packing oil, the average iodine number of the natural oil of experimental packs of Maine sardines, and the volume of natural oil exuded in processing the particular sample. However, the calculated iodine number was as often above as below the determined value, and the differences were in general greater than those between the iodine number of the packing oil and the drained oil (Tables 1 and 2). Table 4 gives a single example of the method of calculation where the agreement was reasonably good. Under the State regulation, which is rigidly enforced, the assumption that the average added oil per can is 20 ml. is justified, though no doubt there would be some variation from can to can. Another factor is the variation



in iodine number of the natural sardine oil. A third variable may result from the method of determining the iodine number of the natural sardine oil. Experimental packs, without added oil, were put up and only the drained oil was taken for the determination. It is possible that the oil retained in the fish might have a somewhat different iodine number from that exuded in the heat process.

TABLE 4.—*Method of calculation*

Average volume of oil recovered from four cans of sardines by draining and extraction.....	23.0 ml.
Average volume of oil assumed to be added at the factory.....	20.0 ml. = 87%
Average volume of natural fish oils in can.....	3.0 ml. = 13%

*Iodine numbers of oils used in calculations*

Olive oil used in packing these sardines.....	83.
Fish oil (from experimental pack, ten cans).....	162.
Iodine number due to olive oil fraction.....	$87\% \times 83 = 72.2$
Iodine number due to fish oil fraction.....	$13\% \times 162 = 21.1$
Calculated predicted iodine number.....	93.3
Actual determined iodine number of this oil mixture.....	93.6

## CONCLUSIONS

Differences between the Hanus iodine number and the refractive index at 25°C. of the oil used in packing Maine sardines and the corresponding constant of the oil drained from the finished product are small.

Adulteration of olive oil used in packing Maine sardines can therefore be detected by determining the constants of the oil drained from the sardines.

# MICROSCOPIC IDENTIFICATION OF STRONTIUM, AMMONIUM, COPPER, AND ZINC BY MEANS OF THEIR CRYSTALLINE PICROLONATES

By WILLIAM V. EISENBERG and GEORGE L. KEENAN (Microanalytical Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

In a recent paper<sup>1</sup> the authors described a microscopic method for the identification of sodium and potassium, which consisted briefly in the formation of characteristic crystalline picrolonates of these cations and a determination of their optical-crystallographic properties. This method has also been found applicable to the detection of strontium, ammonium, copper, and zinc.

<sup>1</sup> *This Journal*, 27, 177 (1944).

The crystalline picrolonates are formed by adding a drop of 0.5 per cent picrolonic acid in 50 per cent alcohol to a small fragment of the unknown material or to a neutral or very slightly acid drop of the test solution on a glass slide. Warming is usually unnecessary. Each of the cations is identified by its characteristic crystalline picrolonate, which forms immediately and is readily visible at  $200\times$  (Figs. 1, 2, 3). In order to identify these precipitates the characteristic crystalline habits are observed, and their significant microscopic-crystallographic properties determined. After the crystalline habit is observed the precipitate on the slide is allowed to dry

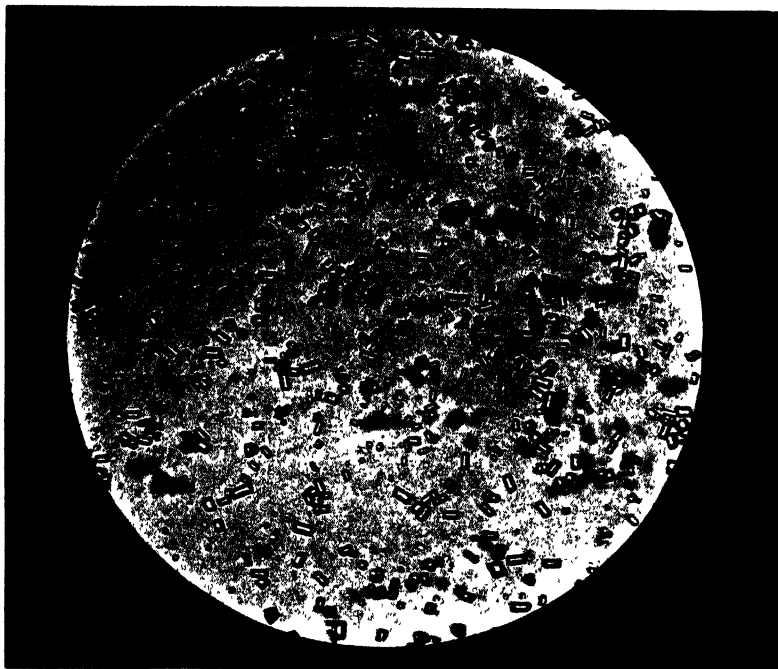


FIG. 1.—CRYSTALS OF STRONTIUM PICROLONATE ( $\times 200$ )

at room temperature and examined with the polarizing microscope, using the immersion method. The characteristic habits and the significant optical-crystallographic data for the picrolonates of strontium, potassium, sodium, ammonium, copper, and zinc are tabulated below in ascending order of the minimum refractive index.

The test for strontium is given even in the presence of relatively large concentrations of  $\text{Ba}^{++}$  and  $\text{Ca}^{++}$ , and similarly the typical crystals of ammonium picrolonate can be demonstrated in the presence of relatively large concentrations of  $\text{Na}^+$  and  $\text{K}^+$ . The characteristic bifurcated rods of ammonium picrolonate, however, appear more slowly

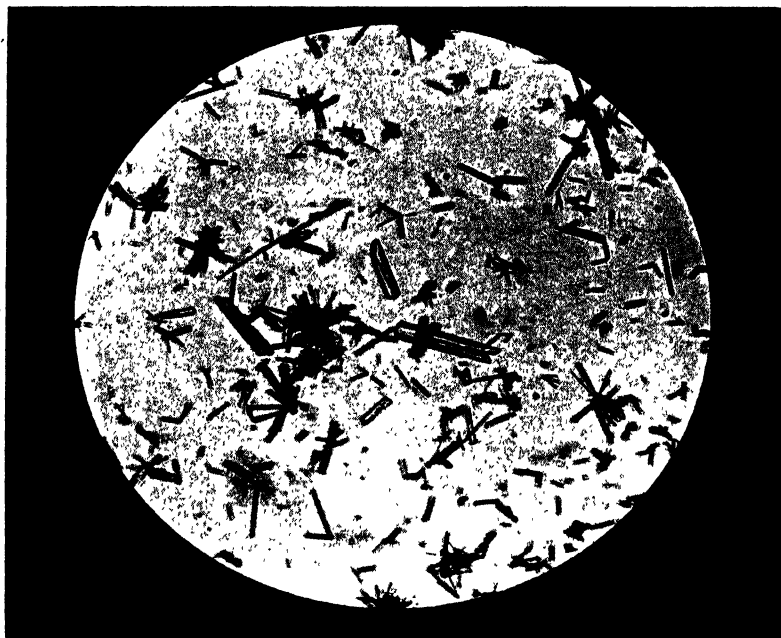


FIG. 2.—CRYSTALS OF AMMONIUM PICROLONATE ( $\times 200$ )



FIG. 3.—CRYSTALS OF COPPER PICROLONATE ( $\times 200$ )

*Significant optical data for strontium, potassium, sodium, ammonium, copper, and zinc picrolonates*

SUBSTANCE	HABIT	$n_{\alpha}$	$n_{\beta}$	$n_{\gamma}$	EXTINCTION	ELONGATION
Sr	Tabular prisms resembling envelope and rooftop forms	1.448	—	>1.734	Inclined	Negative
K	Yellow needles, rods	1.505	1.519	>1.734	Inclined	Negative
Na	Yellow needles, rods	1.616	—	>1.734	Parallel	Negative
NH <sub>4</sub>	Yellow rods, many bifurcated	1.644	1.734	>1.734	Parallel	Positive
Zn	Yellow needles, rods	1.650	1.734	>1.734	Parallel	Positive
Cu	Yellow needles, rods	1.734	—	>1.734	Parallel	Positive

in mixtures. The test for copper is very sensitive even in the presence of other elements, while the sensitivity of zinc in mixtures is somewhat similar to that of ammonium.

The value of the method for complex mixtures may be limited especially where reliance is placed wholly on habit, since habit may be modified by the presence of impurities. From the standpoint of crystalline habit the ammonium and strontium salts are quite characteristic and yield their significant forms even in the presence of other cations. The rosette aggregates of needles of the picrolonates of copper, zinc, sodium, and potassium are similar to each other, and therefore reliance for specific identification must be placed on the determination of the optical-crystallographic properties. With complex mixtures, where a conglomeration of precipitates may form on the slide, studies should be made of the optical properties of those portions of the dried material where individual crystals can be separated by the eye. Crystals of the strontium and ammonium salts can be distinguished easily from other precipitated salts. The identification of sodium, potassium, copper, and zinc, however, will require optical-crystallographic determinations on a number of needles before the identity of the respective picrolonates can be confirmed. Needles exhibiting inclined extinction will immediately indicate potassium, as a glance at the table will show. Those showing parallel extinction indicate copper, sodium, and zinc, and these may be further differentiated by the sign of elongation, which is negative for sodium and positive for copper and zinc. Copper and zinc are further distinguished by their refractive indices.

The procedure described above is most effective on salts of these cations present in dry physical mixtures encountered in food and drug products, where the unknown material may be isolated as minute frag-

ments under a low-power, wide-field, binocular microscope. This offers a quick, direct method for the positive identification of strontium, potassium, sodium, ammonium, copper, and zinc ions.

#### SUMMARY

A chemical microscopic test for the identification of strontium, ammonium, copper, and zinc is described, and optical-crystallographic constants are given for differentiating the strontium, ammonium, copper, and zinc salts formed with picrolonic acid.

### OXIDATION OF ALKANOLAMINES BY PERIODATE

By J. H. JONES (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The selective oxidation by periodate of compounds with hydroxyl groups on adjacent carbon atoms has received thorough study.<sup>1</sup> It has been shown that for many such compounds the reaction is quantitative and fairly rapid. With supporting analyses for the reaction products the reaction may be used to study the structure of polyhydroxy compounds and to determine them quantitatively.<sup>1</sup>

Nicolet and Shinn<sup>2</sup> pointed out that certain  $\alpha$ -hydroxy amines are also selectively oxidized by periodate and applied this fact to the determination of some naturally occurring compounds.<sup>3,4</sup> In their original communication,<sup>2</sup> they reported that diethanolamine reacts rapidly with periodic acid to liberate four mols of formic acid (this reaction could not be confirmed) and that diethylaminoethanol does not react with periodate. A number of aliphatic hydroxy amines (alkanolamines), including 2-aminoethanol, the simplest member of the series, are commercially available, but no further study of the periodate reaction as applied to the alkanolamines appears to have been made. Since alkanolamines are frequently encountered in the products examined in this laboratory an investigation of the oxidation of several such compounds by periodate was undertaken.

All commercially available alkanolamines are  $\alpha$ -hydroxy amines. Unless the alkanolamine is of this type no reaction with periodate would be anticipated.

At room temperature dilute solutions of the primary and secondary alkanolamines are readily oxidized by periodate. In these oxidations an integral number of mols of periodate reacts in a moderate period of time and the additional consumption of periodate over an extended period is negligible.

<sup>1</sup> "Organic Reactions," Vol. II, Chapter 8, John Wiley and Sons, Inc., N.Y. (1944).

<sup>2</sup> *J. Am. Chem. Soc.*, **61**, 1615 (1939).

<sup>3</sup> *J. Biol. Chem.*, **138**, 91 (1941).

<sup>4</sup> *Ibid.*, **139**, 687 (1941).

The reactions may be represented by the following equations:

- (1)  $\text{HOC}_2\text{H}_4\text{NH}_2 + \text{HIO}_4 = 2\text{HCHO} + \text{NH}_3 + \text{HIO}_3$
- (2)  $(\text{HOC}_2\text{H}_4)_2\text{NH} + 2\text{HIO}_4 = 4\text{HCHO} + \text{NH}_3 + 2\text{HIO}_3$
- (3)  $\text{CH}_3\text{CHOHCH}_2\text{NH}_2 + \text{HIO}_4 = \text{HCHO} + \text{CH}_3\text{CHO} + \text{NH}_3 + \text{HIO}_3$
- (4)  $(\text{CH}_3\text{CHOHCH}_2)_2\text{NH} + 2\text{HIO}_4 = 2\text{HCHO} + 2\text{CH}_3\text{CHO} + \text{NH}_3 + 2\text{HIO}_3$
- (5)  $(\text{CH}_3)_2\text{CNHCH}_2\text{OH} + \text{HIO}_4 = \text{HCHO} + (\text{CH}_3)_2\text{CO} + \text{NH}_3 + \text{HIO}_3$
- (6)  $\text{CH}_3\text{OHCNH}_2(\text{CH}_3)\text{CH}_2\text{OH} + 2\text{HIO}_4 = 2\text{HCHO} + \text{CH}_3\text{COOH} + \text{NH}_3 + 2\text{HIO}_3$

Primary alkanolamines react with the same number of mols of periodate as do the analogous polyhydroxy compounds, and the reaction products are those given by the corresponding polyhydroxy compound, plus ammonia.

The data presented here indicate that both diethanolamine and diisopropanolamine react with two mols of periodate to give four mols of aldehyde and one of ammonia. Nicolet and Shinn<sup>2</sup> state that diethanolamine reacts rapidly with periodic acid to give four mols of formic acid. To produce four mols of formic acid from one mol of diethanolamine six mols of periodate would be required. In the experiments performed in this laboratory the consumption of periodate in excess of two mols per mol of diethanolamine was slight unless a large excess of periodate was used. Even when ten mols of periodic acid was used the rate of oxidation after two mols had reacted was slow, and the total consumption of oxidant after 24 hours was less than three mols.

Neither triethanolamine nor triisopropanolamine reacts at a significant rate with periodate. This is in agreement with the observation of Nicolet and Shinn<sup>2</sup> on the oxidation of diethylaminoethanol. It would appear, therefore, that tertiary alkanolamines do not react with periodate.

The reaction products formed by the action of periodate on polyhydroxy compounds may be predicted by assuming that two protons and two electrons are removed from hydroxyls attached to adjacent carbon atoms and that the unstable intermediate compound produced rearranges to a more stable configuration. A similar mechanism may be used to explain the reactions reported here since the supposed intermediate compounds would be readily hydrolyzable to ammonia and the organic compounds indicated by the above equations. For the secondary amines a stepwise reaction may be assumed, i.e., the initial products are two mols of aldehyde and one of the primary amine. The failure of the tertiary amines to react would be explained by the fact that there is no hydrogen attached to the nitrogen atom.

In the experiments described below a substantial excess of periodate was used. Other experiments showed, however, that an excess of 0.1–0.2 mol of periodate is sufficient for quantitative oxidation when a reaction period of 16 hours (overnight) is allowed.

Since the reactions are quantitative they may be used for the determination and identification of the individual amines. If the reaction prod-

ucts are also determined, a quantitative determination of the individual components in certain mixtures of alkanolamines, or alkanolamines and polyhydroxy compounds, is possible. A study of this topic is in progress.

The preparation of the amine hydrochlorides for use as reference standards has been described in a separate report.<sup>5</sup> They were selected for this purpose because they are nonhygroscopic solids and readily purified by recrystallization. The hydrochlorides are neutral or just acid to methyl red, and if a periodate salt neutral to this indicator is used as the oxidant, the formation of an acid as one of the reaction products is easily detected. The presence of the chloride ion does not affect the oxidation or any of the determinations involved.

### EXPERIMENTAL

The results for oxidant consumed, aldehyde produced, etc., are calculated as mols per mol of amine originally present, unless otherwise specified. All determinations of aldehyde, acid, and ammonia were made at a time when the quantity of periodate reduced was that required by the equations above.

*Oxidation of Monoethanolamine (2-Aminoethanol) with Potassium Metaperiodate.*—To 115.2 mg. of monoethanolamine hydrochloride dissolved in water, 100 ml. of 0.0201 *M* potassium metaperiodate (1.7 mols) was added, and the solution was diluted to exactly 200 ml. After one hour, titration of the excess periodate in an aliquot indicated that 0.98 mole of oxidant had been consumed. Overnight the consumption of periodate increased to 1.00 mol and no further increase was noted after three days. Determination of the total aldehyde in an aliquot by the method described by Shupe<sup>6</sup> showed that 1.98 mols of aldehyde had been formed. A 20 ml. aliquot of the solution was transferred to a semi-micro Kjeldahl distillation apparatus and diluted to approximately 30 ml.; 5 ml. of (1+1) sodium hydroxide was added; and the mixture was steam-distilled at a rate of 1.5 ml. per minute. About 20 ml. of distillate was collected in a receiver charged with 2 ml. of 4 per cent boric acid and titrated with 0.02 *N* acid. The recovery of ammonia was 0.99 mol. (The alkanolamines are not appreciably volatile with steam.)

To a portion of the oxidized solution an equal volume of a saturated aqueous solution of dimethyldihydroresorcinol, "dimedon," was added; a crystalline precipitate formed, which melted at 190°C. after a single recrystallization from alcohol. The reported melting point of formaldehyde dimethone is 191.4°C.<sup>7</sup>

*Oxidation of Monoethanolamine with Periodic Acid.*—To 334.2 mg. of monoethanolamine hydrochloride, dissolved in water, 10 ml. of 0.490 *M*

<sup>5</sup> See p. 467.

<sup>6</sup> *This Journal*, 26, 249 (1943).

<sup>7</sup> *Z. Anal. Chem.*, 77, 241, 321 (1929)

periodic acid (1.4 mols) was added, and the solution was diluted to exactly 50 ml. The oxidant consumed in one hour was 0.96 mol; in 24 hours it was 1.01 mols.

*Oxidation of Diethanolamine with Potassium Metaperiodate.*—No pure compound suitable for a reference standard was available. A master solution was prepared from a commercial sample of the amine, and the amine content was determined by titration with standard acid. An aliquot of this solution containing 54.1 mg. of diethanolamine was exactly neutralized to methyl red with 0.05 *N* acid, 100 ml. of 0.0204 *M* potassium metaperiodate was added (4.0 mols), and the solution was diluted to exactly 200 ml. After one hour 2.00 mols of oxidant had been consumed but no acid was produced. No further increase in the amount of periodate reduced was noted after three days, and at no time was there evidence of the formation of any formic acid. Analysis of an aliquot by Shupe's procedure indicated that 3.98 mols of aldehyde had been produced. The ammonia recovered by steam distillation was 0.99 mol.

*Oxidation of Diethanolamine with Periodic Acid.*—To a solution containing 108.2 mg. of diethanolamine, 10 ml. of 0.490 *M* periodic acid (4.7 mols) was added, and the mixture was diluted to 100 ml. in a volumetric flask. The periodate reduced in one hour was 1.95 mols; in 24 hours it was 2.03 mols. The consumption of periodate continued to increase slowly and after four days amounted to 2.2 mols.

*Oxidation of Isopropanolamine (1-Amino-2-Propanol) with Potassium Metaperiodate.*—To 122.2 mg. of isopropanolamine hydrochloride dissolved in water, 100 ml. of 0.0204 *M* potassium metaperiodate (1.9 mols) was added, and the solution was diluted to exactly 200 ml. After two hours a titration of an aliquot indicated a consumption of 0.99 mol of oxidant. Overnight the periodate consumed increased to 1.00 mol, and no further increase was noted in four days. The total aldehyde produced amounted to 2.00 mols, and a determination of acetaldehyde by the method of Nicolet and Shinn<sup>3,8</sup> indicated that 0.98 mol of this substance had been formed. Determination of ammonia by the method outlined above gave a recovery of 0.99 mol.

*Oxidation of Diisopropanolamine with Potassium Metaperiodate.*—To 95.7 mg. of diisopropanolamine hydrochloride, dissolved in water, 100 ml. of 0.0204 *M* potassium metaperiodate (3.6 mols) was added, and the mixture was diluted to 200 ml. in a volumetric flask. After two hours the periodate consumed was 1.98 mols; overnight the consumption increased to 2.00 mols, and the additional consumption in eight days was negligible. No acid was formed. The total aldehyde and acetaldehyde produced amounted to 3.91 and 1.98 mols, respectively. The recovery of ammonia was 0.99 mol.

<sup>8</sup> *J. Am. Chem. Soc.*, 63, 1456 (1941).



*Oxidation of 2-Amino-2-Methyl-1-Propanol with Potassium Metaperiodate.*—To 95.7 mg. of 2-amino-2-methyl-1-propanol hydrochloride, dissolved in water, 100 ml. of 0.0201 *M* potassium metaperiodate (2.7 mols) was added, and the solution was diluted to exactly 200 ml. After 90 minutes, titration of an aliquot indicated a consumption of 0.99 mol of periodate, and overnight the consumption of oxidant increased to 1.00 mol. The recovery of formaldehyde dimethone from an aliquot of this solution was 96 per cent of the calculated amount (if one mol of formaldehyde was produced in the reaction). The ammonia recovered amounted to 0.98 mol.

*Oxidation of 2-Amino-2-Methyl-1, 3-Propanediol with Potassium Metaperiodate.*—To 67.6 mg. of 2-amino-2-methyl-1, 3-propanediol hydrochloride, dissolved in water, a drop of methyl red was added, and the solution was made basic to this indicator by the addition of 0.05 ml. of 0.02 *N* sodium hydroxide; 100 ml. of 0.0204 *M* potassium metaperiodate (4.3 mols) was added; and the solution was diluted to exactly 200 ml. The solution became more acid immediately, and in 90 minutes 1.96 mols of oxidant had been consumed. Overnight the periodate consumed increased to 2.01 mols, and after three days the consumption of oxidant was 2.03 mols. The acid liberated could not be accurately titrated with methyl red as an indicator, but the titration did indicate that more than 0.8 mol of acid was produced. The recovery of ammonia was 1.00 mol and 1.99 mols of aldehyde was formed.

*Oxidation of 2-Amino-2-Methyl-1, 3-Propanediol with Sodium Metaperiodate and Determination of Acid Produced.*—To 106.7 mg. of 2-amino-2-methyl-1, 3-propanediol hydrochloride, dissolved in water, 20 ml. of 0.965 *M* sodium metaperiodate (2.6 mols) was added, and the solution was diluted to exactly 100 ml. After 16 hours, titration of the excess periodate in an aliquot of the solution indicated that 1.99 mols of oxidant had reacted. To 50 ml. of the oxidized solution 0.2 gram of ethylene glycol was added to destroy the excess periodate, and after 15 minutes the solution was titrated with 0.0540 *N* sodium hydroxide, a glass electrode *pH* meter being used to follow the change in *pH*. If a *pH* of 7 at the equivalent point is assumed, 6.95 ml. of the base was required for the neutralization. This titration corresponds to the production of 1.00 mole of acid from each mol of the amine.

The "blank" on 10 ml. of the sodium metaperiodate solution in 50 ml. of water, after reduction with 0.2 gram of ethylene glycol, was negligible.

#### SUMMARY

A study has been made of the selective oxidation of  $\alpha$ -alkanolamines by periodate. Primary and secondary alkanolamines are quantitatively oxidized in a moderate period of time but the tertiary amines do not react at a significant rate.

Equations are given for the reaction of typical alkanolamines with periodate, as well as data showing agreement between theory and experimental results.

## PREPARATION OF ALKANOLAMINE HYDROCHLORIDES

By J. H. JONES (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

Recently the author investigated reactions and procedures applicable to the identification and determination of the so-called "amine emulsifiers." In part of this work<sup>1,2</sup> it was advantageous to use the hydrochlorides of the commercially available alkanolamines as reference compounds.

Triethanolamine hydrochloride is easily prepared from hydrochloric acid and an alcoholic solution of the amine, but attempts to make other alkanolamine hydrochlorides by the same procedure were not successful. The preparation of some alkanolamine hydrochlorides has been reported in the literature, but the methods used seem inconvenient and do not appear to give pure compounds. Methods for the preparation and purification of the hydrochlorides were therefore investigated.

### PREVIOUS WORK

Triethanolamine hydrochloride, which is only slightly soluble in ethyl alcohol, can be prepared by the addition of hydrochloric acid to an alcoholic solution of the amine.<sup>3</sup> This hydrochloride has been used by Eastland *et al.*, for the identification and quantitative determination of the amine,<sup>4</sup> and by Germann and Knight<sup>5</sup> as an intermediate in the preparation of pure triethanolamine.

Wurtz<sup>6</sup> obtained an impure sample of monoethanolamine hydrochloride by evaporation of a solution of the salt to a thick sirup, which, after standing a long time, deposited fine crystals which melted "below 100°C." Wurtz states that his analysis indicated the formula  $C_2H_5ONCl$ , but he gives no analytical data. Gabriel<sup>7</sup> prepared the hydrobromide in a similar manner and reported that it also melts "below 100°C." and is hygroscopic. No report of a purer preparation of either of these compounds could be found in the literature.

The hydrochlorides of 1-amino-2-propanol and 2-amino-1-propanol were also prepared by Gabriel.<sup>7,8</sup> He evaporated a solution of the hydro-

<sup>1</sup> *This Journal*, 27, 309 (1944).

<sup>2</sup> See p. 462.

<sup>3</sup> *J. Am. Chem. Soc.*, 55, 4150 (1933).

<sup>4</sup> *Analyst*, 62, 261 (1937).

<sup>5</sup> *Ann.*, 121, 228 (1862).

<sup>6</sup> *Ber.* 21, 566 (1888).

<sup>7</sup> *Ibid.*, 49, 2120 (1916).

<sup>8</sup> *Ibid.*, 50, 804 (1917).

chloride to a sirup under vacuum, dissolved the sirup in alcohol, and precipitated the salt with ethyl acetate. The 2-amino-1-propanol hydrochloride was reported to be very hygroscopic.

The preparation of triisopropanolamine hydrochloride has been reported by Krasuskii.<sup>9</sup> This compound was purified by recrystallization from absolute alcohol.

Piloty and Ruff<sup>10,11</sup> obtained the hydrochlorides of 2-amino-1, 3-propanediol and 2-amino-2-methyl-1, 3-propanediol by dehydration of a sirup the salt over sulfuric acid in a vacuum and reported that these compounds were hygroscopic.

#### EXPERIMENTAL

Samples of the alkanolamines obtained from commercial sources were used without further purification for the preparation of their hydrochlorides.

*Monoethanolamine (2-Aminoethanol) Hydrochloride.*—Ten grams of monoethanolamine was placed in a small flask and dissolved in 50 ml. of *n*-propanol. The container was immersed in cold water to absorb the heat developed in the neutralization, and dry hydrogen chloride was passed through the solution until a slight excess was present. (The completion of the neutralization was checked with congo red test paper.) A copious precipitate formed toward the end of the neutralization. Any lumps of crystals were broken up. The precipitate was left in contact with the solvent for about 30 minutes, collected on a Büchner funnel, and washed with a little *n*-propanol and several portions of acetone. The filtrate was then concentrated to 25 ml., cooled, and seeded. This produced a second small crop of crystals. The crude material was purified by recrystallization from absolute alcohol (5 ml. per gram) or by the method given below under monoisopropanolamine hydrochloride. A single recrystallization by either method usually gave a pure compound.

*Monoisopropanolamine (1-Amino-2-Propanol) Hydrochloride.*—Ten grams of the amine in 50 ml. of *n*-propanol was neutralized with dry hydrogen chloride as described above. The neutralized solution was boiled, cooled, seeded, and set aside for a few hours. The precipitate was crushed and stirred frequently during this period to hasten complete crystallization. The precipitated hydrochloride was then filtered and washed with acetone. The filtrate was evaporated to 25 ml., cooled, seeded, and diluted with 25 ml. of acetone. A second crop of crystals was thus collected. The crude material was dissolved in hot absolute alcohol (5 ml. per gram), the solution was cooled, seed crystals were added, and the hydrochloride was precipitated by slow addition of several volumes of acetone. The solution was vigorously stirred throughout the addition of the acetone to hasten

<sup>9</sup> *J. Gen. Chem.* (U.S.S.R.), 6, 460 (1936); *C.A.*, 30, 6377 (1936).

<sup>10</sup> *Ber.*, 30, 1656 (1897).

<sup>11</sup> *Ibid.*, 2057.

crystallization and prevent the separation of the hydrochloride as an oil. One re-precipitation usually produced a pure compound.

In the absence of seed crystals this compound crystallized very slowly from *n*-propanol. For the initial supply of seed crystals, a small portion of the *n*-propanol solution of the hydrochloride was heated at 100°–105°C. until the *n*-propanol had been expelled. The sirupy residue, placed in a vacuum desiccator over calcium chloride, crystallized after several hours to a solid mass. This solid material was covered with acetone and crushed, and a portion of the crystals was used for seeding purposes. Subsequent preparations were seeded with purified material obtained from previous experiments.

*2-Amino-2-Methyl-1-Propanol Hydrochloride.*—Ten grams of 2-amino-2-methyl-1-propanol in 50 ml. of *n*-propanol was neutralized with dry hydrogen chloride. A precipitate of the hydrochloride usually formed when neutralization was complete. A portion of this precipitate was reserved for use as seed crystals unless material from a previous experiment was available. The remainder of the solution containing the precipitate was heated to boiling and boiled for several minutes. (The precipitate dissolved completely.) After the solution had cooled, it was seeded, stirred until crystallization began, and set aside for about an hour. The precipitated hydrochloride was filtered off, the solvent was removed as completely as possible by the application of strong suction, and the crude material was washed with several portions of acetone. A second crop of crystals was collected by evaporation of the filtrate to 25 ml. and dilution with 25 ml. of acetone. Occasionally a third crop of crystalline material could be obtained by a repetition of this process. The crude product was purified by the procedure used for the purification of monoisopropanolamine hydrochloride.

*2-Amino-2-Methyl-1, 3-Propanediol Hydrochloride.*—This compound was prepared and purified by the procedure described under 2-amino-2-methyl-1-propanol hydrochloride. The original amine was colored bright yellow and the crude hydrochloride a yellowish white. A pure white product was obtained when "Norit" decolorizing carbon was used in the purification.

*Triisopropanolamine Hydrochloride.*—The hydrochloride was prepared by the method used to obtain 2-amino-2-methyl-1-propanol hydrochloride and purified by recrystallization from absolute alcohol (5 ml. per gram) or by precipitation from absolute alcohol with acetone.

*Diisopropanolamine Hydrochloride.*—Ten grams of the amine in 30–40 ml. of *n*-propanol was neutralized with dry hydrogen chloride. The solution was evaporated to 25 ml. and cooled, 50 ml. of acetone was added, and the precipitated oil was stirred at intervals until crystallization was induced. (The addition of seed crystals did not appreciably increase the rate of crystallization.) After the crystalline material had stood overnight it was filtered off and washed thoroughly with acetone. The crude product

was purified by repeated extraction of the solid material with cold acetone.

Typical yields and analytical data on the compounds prepared are given in Table 1. In all experiments where the yield was determined the crude products were dried for several hours over calcium chloride in a vacuum desiccator to remove the adhering solvent.

TABLE 1.—*Alkanolamine hydrochlorides*

HYDROCHLORIDE OF—	YIELD <sup>a</sup>	MELTING POINT <sup>b</sup>	CHLORINE	
			FOUND	CALCULATED
	<i>per cent</i>	<i>°C.</i>	<i>per cent</i>	<i>per cent</i>
Monoethanolamine (2-aminoethanol)	90–95	82–83°	36.40	36.35
Monoisopropanolamine (1-amino-2-propanol)	65–70	72–73 <sup>d</sup>	31.82	31.78
Diisopropanolamine	65–70	79–82°	20.93	20.91
Triisopropanolamine	70–75	209–211°	15.63	15.57
2-Amino-2-methyl-1-propanol	65–70	203–206°	28.20	28.23
2-Amino-2-methyl-1, 3-propanediol	70–80	90–91 <sup>f</sup>	25.02	25.04

<sup>a</sup> Crude product.

<sup>b</sup> All melting points were determined on a melting-point block. As noted by others, melting points determined in this manner are usually 1°–2°C. lower than those determined in a capillary tube.

<sup>c</sup> Apparently no melting points have been recorded for these compounds.

<sup>d</sup> Previously reported melting point, 72.5°–74°C.<sup>8</sup>

<sup>e</sup> The melting point and analysis of this compound indicate a much purer product than was obtained by Krasuski,<sup>9</sup> who reported a melting point of 186°–188°C. for his preparation.

<sup>f</sup> Previously recorded melting point, 91°–92°C.<sup>11</sup>

## DISCUSSION

The alkanolamine hydrochlorides are apparently miscible with water, and with the exception of triethanolamine hydrochloride they are not precipitated as crystalline solids by the addition of hydrochloric acid to a solution of the amine in water or ethyl alcohol. Most of the alkanolamines are not sufficiently soluble in ether, benzene, etc., to allow the preparation of any appreciable quantity of the hydrochlorides by precipitation from these solvents with hydrogen chloride. However, the hydrochlorides may be conveniently prepared by neutralization of a *n*-propanol solution of the amine with dry hydrogen chloride. The use of *n*-propanol as the solvent in this procedure has the following advantages:

(1) The alkanolamines are very soluble in *n*-propanol.

(2) Most of the hydrochlorides are only moderately soluble in this solvent and precipitate from it as crystalline solids.

(3) A higher concentration of water may be present without separation of an alkanolamine hydrochloride-water layer. (When such a layer separates it is usually impossible to recover conveniently the solid hydrochloride.)

(4) The impurities present in commercial samples of the amines appear to be quite soluble in *n*-propanol.

(5) The crude product obtained is substantially purer than that produced by other methods of preparation, and can readily be further purified.

*n*-Propanol can also be advantageously used in the isolation of alkanolamine hydrochlorides from aqueous solutions. The sirups obtained by the evaporation of aqueous solutions of the hydrochlorides are soluble in boiling *n*-propanol, and one or two evaporations with this solvent usually dehydrate the mixture sufficiently (presumably by the formation of the constant boiling mixture) to allow the hydrochloride to separate as a crystalline solid. This procedure does not give as high yields as the above method, but it has proved useful in the isolation of the amine hydrochlorides from solutions or emulsions for identification.

Diethanolamine hydrochloride could not be prepared as a crystalline solid by any method tried. In every experiment this compound separated from the solution as an oil and crystallization could not be induced.

In the preparation and purification of the hydrochlorides supersaturated solutions were frequently encountered and occasionally the compounds precipitated as oils. In most cases crystallization could be induced by vigorous stirring of the solution or by scratching the sides of the vessel, but the addition of seed crystals was even more effective. Such seed crystals for all the compounds listed in Table 1 may be prepared by the procedure used for monoisopropanolamine hydrochloride.

Contrary to the impression gained from the comments in the literature the pure crystalline hydrochlorides are not hygroscopic. Exposure of the pure salts to ordinary atmospheric conditions for several hours caused no gain in weight. Therefore it is not necessary to exclude atmospheric moisture while samples for analysis are weighed.

Solutions of the hydrochlorides, varying in strength from 0.2 to 0.002 *M* were found to be neutral or just acid to methyl red. Thus a solution of 0.2 gram of 2-amino-2-methyl-1-propanol in 50 ml. of distilled water was acid to this indicator, but the addition of 0.1 ml. of 0.02 *N* sodium hydroxide produced a yellow solution. Methyl red, therefore, is a suitable indicator for the titration of alkanolamines with strong acids.

The hydrochlorides have proved useful as derivatives for the identification of the amines. If the melting point alone does not provide positive identification the hydrochlorides may be analyzed by simple procedures. The periodate reaction<sup>2</sup> is particularly useful for this purpose since it gives information as to the structure of the amine.

#### SUMMARY

Methods for the preparation and purification of several alkanolamine hydrochlorides have been presented. Analytical data for the compounds

are given. Contrary to earlier reports in the literature these hydrochlorides are not hygroscopic. They have proved useful as reference compounds and for the identification of the amines.

## DETERMINATION OF SODIUM ACETATE IN FD&C BLUE NO. 1

By R. N. SCLAR and G. R. CLARK (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The Coal-Tar Color Regulations (S.R.A.F.D.C.3) limit the quantity of sodium acetate permitted as an impurity in FD&C Blue No. 1 to not more than 3.0 per cent.

The tentative A.O.A.C. method<sup>1</sup> for the determination of sodium acetate in FD&C Blue No. 1 is time consuming and gives varying results. The procedure described here, which is based on Freudenberg's method<sup>2</sup> for the determination of acetyl groups, gives more satisfactory results.

The sodium acetate is esterified to ethyl acetate in the presence of *p*-toluenesulfonic acid and silver toluenesulfonate, and distilled. The ester is saponified with a measured excess of standard sodium hydroxide, and the unconsumed base is titrated with standard acid.

The silver toluenesulfonate, by precipitating silver halides, prevents interference by any halogen salts present.

### METHOD

#### REAGENTS

*p*-Toluene sulfonic acid.

*Silver toluenesulfonate*.—Dissolve freshly prepared and washed  $\text{Ag}_2\text{O}$  in approximately 10% excess of *p*-toluenesulfonic acid solution, evaporate to dryness, and dry at 135°C. for 8 hours.

#### APPARATUS

The apparatus can be assembled from stock items. The distilling flask is a 125 ml. acetylation flask with 24/40 standard taper joint. The receiver is a 300 ml. flask. The assembled apparatus is shown in Figure 1.

#### PROCEDURE

Add 20 ml. of absolute alcohol to the receiving flask, place it in an ice bath, and connect it to the apparatus.

In the distilling flask, place a 2 gram sample of the dye, 4 grams of *p*-toluenesulfonic acid, 1 gram of silver toluenesulfonate, and 40 ml. of absolute alcohol. Mark the flask to show the level of the contents.

Distil from a vigorously boiling water bath; 15 minutes after the first distillate drops into the receiver, add 20 ml. of absolute alcohol and distil for 10 minutes; then add enough absolute alcohol to bring the contents to the original level and again distil for 15 minutes.

Disconnect the receiving flask from the apparatus, add a measured excess of 0.1 *N* NaOH, connect to a reflux condenser, and reflux for 10 minutes. Wash down the

<sup>1</sup> *Methods of Analysis, A.O.A.C.*, 1940, 255.

<sup>2</sup> Freudenberg, K. and Harder, M., *Ann.*, **433**, 230 (1923); **494**, 68 (1932).

condenser with water, remove the flask, and add enough distilled water to make an approximately (1+1) water-alcohol mixture. Cool to room temperature and titrate with 0.1 *N* HCl and phenolphthalein indicator.

Run blank distillation on all reagents, using the same procedure but omitting the dye sample.

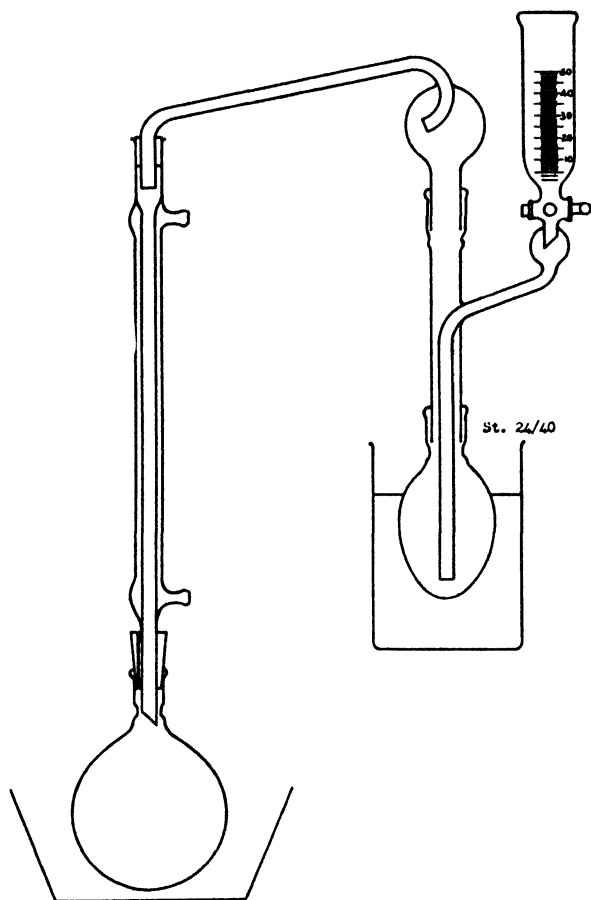


FIG. 1

From the net volume of standard NaOH solution required for hydrolysis of the ester, calculate the amount of sodium acetate in the sample.

1 ml. of 0.1 *N* NaOH = 0.0082 gram of  $C_2H_3O_2Na$ .

Results by this method are shown in Table 1. Several determinations of the blank were run on the reagents used. The blank, which varied from 0.50 to 0.54 ml. of 0.1 *N* HCl, is equal to 0.20–0.22 per cent of sodium acetate in a 2 gram sample.



TABLE 1.—*Recovery of sodium acetate in presence of FD&C Blue No. 1*

NO.	ADDED	FOUND	CALCULATED TOTAL	DIFFERENCE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
—	0	0.08	—	—
—	0	0.09	—	—
—	0	0.10	—	—
—	0	0.21	—	—
<hr/>				
		Av. 0.12*		
1	6.58	6.57	6.70	— .13
2	3.54	3.54	3.66	— .12
3	3.51	3.49	3.63	— .14
4	2.65	2.68	2.77	— .09
5	2.56	2.68	2.68	.00
6	2.75	2.82	2.87	— .05
7	2.62	2.70	2.74	— .04
8	2.63	2.64	2.75	— .11
9	2.52	2.59	2.64	— .05
10	2.52	2.58	2.64	— .06

\* The average quantity of the sodium acetate actually present in this batch. Deductions for reagent blanks were made.

An average difference of — .08 per cent was obtained between the found and calculated values of sodium acetate in ten determinations when 2 gram aliquot samples of FD&C Blue No. 1 were used.

#### SUMMARY

A method for determining sodium acetate in FD&C Blue No. 1 has been described. Typical results are given.

## BOOK REVIEWS

**Semimicro Quantitative Organic Analysis.** By E. P. CLARK. Academic Press, Inc., New York, 1943. 135 pp. Price \$2.50.

This book presents a description of methods for the elementary organic analyses most frequently encountered in a research laboratory, many of which have been for several years the official methods of the Association of Official Agricultural Chemists. This is perhaps the first American publication dealing with the analysis of semimicro quantities that gives detailed dimensional and pictorial representations of suitable apparatus which, for the most part, can be fabricated from the material available in the average organic research organization. This book could not only serve as a text for classroom instruction, but it is also especially valuable as a reference for the advanced analyst. It is particularly well adapted for the worker who, through temperament or lack of proper space or equipment, experiences difficulty with micro procedures.

The first chapter gives a unique treatment of analytical procedure, which is made more valuable by the inclusion of much original material based upon the author's many years of experience. The next eleven chapters present methods for the determination of carbon and hydrogen, Kjeldahl and Dumas nitrogen, halogens, sulfur, phosphorus, molecular weights, volatile fatty acids, and the alkoxyl, acetyl, and carboxyl radicals. Each of these thoroughly tested methods is treated in such a concise and yet sufficiently complete manner that there is never any doubt regarding the correct manipulations to be employed for arriving at a successful conclusion of the analysis.

The final chapter contains many useful reference tables, and of special note is a table containing the percentage composition and molecular weights of a series of carbon-hydrogen-oxygen compounds encountered in natural products.—FRED ACREE, JR.

**Soybean Chemistry and Technology.** By KLARE S. MARKLEY and WARREN H. Goss. Chemical Publishing Company, Inc., Brooklyn 2, New York (1944). 261 pages. Price \$3.50.

This book is presented in two parts, preceded by a brief introduction touching on historical, horticultural, and production topics. The first part on the Chemical Composition of the Soybean and Properties of Constituents and Derived Products includes the following topics: (on composition and properties) mineral constituents, protein and other nitrogenous constituents, enzymes, carbohydrates, glycosides, pigments, vitamins, oil and oil-soluble constituents. There are some 20 pages on physical and chemical characteristics of soybean oils, fatty acids and glycerides, sterols and other unsaponifiable constituents, oil-soluble pigments, antioxidants, and phosphatides.

In Part II, after a brief historical sketch of the processing industry and a few pages on the grading and storage of the beans, the various methods of processing are covered in some detail. There are descriptions, and in many cases illustrations, of the Anderson expeller, the various types of presses, mechanical and hydraulic, and of the numerous continuous solvent extractors, with a word or two on processes involving water flotation after chemical, mechanical, or enzymic disintegration. Full treatment is given to methods of refining the oil and of converting it into forms suitable for shortening and margarine. Other subjects covered include soy flour and soy phosphatides. Brief mention is made of the cost of processing beans, and lists are given of manufacturers of equipment, of mills operating on a full or part time basis, and of mills under construction.

It seems unfortunate that the book contains so very little information on soy flour and soy grist. This totals only a half page under the caption "soy flour."

The material is presented in a clear concise manner, well printed on good paper, and seems to be free of errors. This book, with 477 references cited to the literature under Part I and 207 under Part II, will make a valuable contribution to those interested in the various phases of the soybean industry or in research. Its value is enhanced by the authors' own data and investigations.—V. E. MUNSEY.





DR. L. B. BROUGHTON, 1886-1943

## LEVIN BOWLAND BROUGHTON

Dr. L. B. Broughton, Dean of the College of Arts and Sciences of the University of Maryland, died suddenly at his home in College Park, Maryland, Monday, December 13, 1943. The sentiment of the University community was voiced by the President, Dr. H. C. Byrd, when he stated, "Dr. Broughton is simply irreplaceable. He gave his life to the University of Maryland and the thousands of students who will come here in the years to come will be the beneficiaries of what he has done."

Dr. Broughton was born on the Eastern Shore of Maryland in the town of Pocomoke, March 29, 1886. He attended the Pocomoke High School and Bellefonte Academy at Bellefonte, Pennsylvania. He received his Bachelor of Science degree in chemistry at the Maryland Agricultural College in 1908 and his Master of Science degree in 1911. He pursued his graduate studies at George Washington University, Johns Hopkins University, and Ohio State University. The last-named institution granted him the Doctor of Philosophy degree in 1926.

Dr. Broughton joined the staff of Maryland Agricultural College as an Assistant Chemist in 1908. He was given the title of Associate Chemist in 1914, full Professor in 1918, and was made State Chemist and head of the Chemistry Department in 1929. He succeeded Thomas H. Taliferro as Dean of the College of Arts and Sciences in 1937. Dr. Broughton retained the title of State Chemist to the time of his death, and in recent years was appointed by Governor O'Connor as a Commissioner of the Maryland State Department of Geology, Mining and Water Resources.

In addition to scientific organizations such as the American Chemical Society and the Association of Official Agricultural Chemists, Dean Broughton was a member of several social and civic groups. Among these were Kappa Alpha, a social fraternity; Phi Kappa Phi, honorary scholastic; Sigma Xi, research; Alpha Chi Sigma, professional chemical fraternity; Omicron Delta Kappa, honorary leadership; and the Rotary Club of College Park. He took an active interest in church work and was a member of the Church Committee of St. Andrew's Chapel at College Park and a member of the Brotherhood of St. Andrew, an international organization of churchmen.

On the Maryland campus Dr. Broughton was considered a personal friend by both the students and faculty. He easily ranked as one of the most handsome and best dressed men at the University. Even in the laboratory he retained his immaculate appearance with gold cuff links and a stiff collar on a pure white shirt. His heavy suit of gray hair and his eye glasses held by a long black ribbon added to his distinguished appearance. He was always interested in young people's affairs and retained to the last a lively interest in athletics. For many years he served as chairman of the University Athletic Board.

His human interest in dealing with people was well illustrated by his personal discussions with his associates in the chemistry department. He was always careful to consider the other members of the department as associates and never as subordinates. While head of the Chemistry Department, almost every year at the close of school in June he organized a fishing party for the members of the department, and a glorious day was spent casting for bluefish in Chesapeake Bay. Dr. Broughton loved his native Eastern Shore and endeavored to spend a week or more each summer at Ocean City, Maryland.

Dr. Broughton's greatest interest was in the field of agricultural chemistry, even from the date of his graduation. In 1912 his investigation of lime distribution and loss in the soil was published as a Maryland Experiment Station Bulletin. It was on this research that he was awarded the Master of Science degree. His dissertation


presented for the Doctor of Philosophy degree at Ohio State University, entitled "A Study of the Relation of Fineness to the Efficiency of Limestone in Correcting Soil Acidity," was completed under the direction of Dr. Ferman E. Bear. Among his other publications having direct bearing on agricultural technology were the following:—"The Test of Availability of Different Grades of Limestone," "Potash from Industrial Alcohol," "An Industrial Alcohol By-Product Stock Food," "Studies Relative to the Hydrolysis of the Fat of Home Cured Hams," and "Worm-seed Oil Production."

In the Presidential Address before the 57th Annual Meeting of the Association of Official Agricultural Chemists, Dr. Broughton stated that he attended his first meeting of the Association in 1908. For thirty-five years he maintained a strong interest in the affairs of the Association. In 1929 and 1930 he served as Associate Referee on *Chenopodium*. This assignment undoubtedly came about because of his interest in the production of worm-seed oil in Maryland and his researches on ascaridol. From 1933 to 1938 he was a member of Subcommittee B and for the last two years of this term was the Chairman. Dr. Broughton's ardent work on this committee earned him a position on the Executive Committee of the Association from 1937 to 1943. He was elected Vice-President of the Association for the two years, 1939 and 1940, and President in 1941. It is a marked occasion when a member serves the Association for such a long time and in such important capacities.

Dr. Broughton's presidential address, presented October 27, 1941, was entitled "The Formation and Development of the Association of Official Agricultural Chemists." This will long stand as a classical review of the development of agricultural chemistry in the Western part of the world and especially in America. In this address he also indicated the progress of instruments as analytical tools.

Dean Broughton is survived by his widow, Mrs. Laurise McDonnell Broughton; a daughter, Mrs. Elnor C. Etienne, and a son, Barnett, who is a cadet at West Point.

CHARLES E. WHITE



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## LIEUTENANT MELVIN MORTON SPRUIELL

On July 6, Mr. and Mrs. J. M. Spruiell, of Leeds, Alabama, received that saddest of all messages from the War Department—"The Secretary of War desires me to express his deepest regrets that your son, First Lieutenant Melvin M. Spruiell, was killed in action on eleven June in France."

Dr. Spruiell was appointed as analyst in the Food and Drug Administration in July, 1939. Being a reserve officer, he was called into active service on January 13, 1942. When the Army called for volunteers for paratroop service, he was among the first to apply. As a member of the 101st Airborne Division, he was sent overseas, where his unit received some nine months special training preparatory to the invasion of France.

Dr. Spruiell received his A.B. degree at Alabama Polytechnic Institute after work in chemical engineering. He received a fellowship to the University of Tennessee, which conferred a Master's degree upon him. Later, again under a fellowship, he took graduate work at Ohio State University and received his Ph.D. in 1939. While assisting in physiological chemistry at Ohio State University he was elected to Sigma Xi, a recognition of the excellence of some of his research work.

While serving in the Food and Drug Administration his unusual talent as a chemist and his splendid qualities as a man immediately became apparent. In addition to analyzing a wide variety of food, drug, and cosmetic products, he concluded a number of original investigations. Especially noteworthy was his work on cerium and on methods for the determination of urea in complex mixtures. He continuously evidenced an insatiable curiosity regarding the different avenues of approach to his laboratory problems and was satisfied with nothing less than absolute accuracy and completeness. His work definitely marked him as a man destined for rapid and far-reaching advancement in the food and drug field.

In an article in the newspaper of Leeds, Alabama, the following paragraph appeared:

Melvin Morton Spruiell gave his life for his country in the Invasion of France, June 11. It was a high price to pay, for his was an outstanding life, one of high morals, clean thoughts, character, unusual intelligence, a keen sense of humor, appreciation of fine arts, and deep religious convictions.

S. A. POSTLE

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## REPORT ON UNFERMENTED REDUCING SUBSTANCES IN MOLASSES

By F. W. ZERBAN (New York Sugar Trade Laboratory,  
New York, N. Y.), *Associate Referee*

Since the results obtained by the collaborators do not check satisfactorily, and it is not known whether the discrepancies are due to faults in the determination of copper reducing power, or to differences in the action of the yeast used by individual collaborators, it is recommended that in further work on this subject the determination of copper reducing power be investigated separately, with samples of commercial distillery slop, and that the study of the effect of the yeast be deferred until that problem has been solved.

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## REPORT ON HONEY AND HONEYDEW HONEY

By GEORGE P. WALTON (Special Commodities Branch,  
Office of Distribution, War Food Administration,  
Washington, D. C.), *Associate Referee*

A study of honeydew honey was started by this Association following its 56th Annual Meeting in 1940. Since among the commonly recognized constituents of this product, its dextrin content appeared to give greatest promise to the chemist for distinguishing honeydew honeys from true nectar honeys and in detecting the presence of honeydew in honeys the collaborative work during 1941 was in part directed towards simplifying and improving the tentative method for determining dextrin in honeys (*Methods of Analyses*, A.O.A.C., 1940, 510).

*Dextrin.*—The results of this collaborative work, which were reported at the 1941 meeting (*This Journal*, 25, 681) proved disappointing. Not only were the data for dextrin content obtained by a proposed new method employing double precipitation with alcohol highly erratic, but the variation in results obtained by the official A.O.A.C. method was also excessive.

The work planned for 1942 projected a continuation of collaborative study of both the tentative and the double precipitation methods for dextrin (*This Journal*, 25, 683), each method to be modified by the requirements that a Gooch crucible be used in place of filter paper in filtering off the alcohol precipitate; that each method be liberalized to permit weighing the charge of honey directly into an Erlenmeyer flask, and that the addition of alcohol be controlled by weighing, instead of by bringing to a given volume. These proposed improvements were in part suggested by M. J. Proffitt, one of the 1941 collaborators.

In line with the proposal to control the addition of alcohol by weighing,

instead of by completing to volume, the Associate Referee experimentally determined weight and volume relationships existing between the charge of honey, the water present, and the alcohol required in the precipitation of dextrin by the official method. A short paper reporting this work has been prepared for publication in *This Journal* (see p. 582). This work shows that in the 95-96 per cent (by volume) alcohol, at 20°C., the charge of 4-8 grams of liquid honey, under the conditions prescribed for the precipitation of the dextrin by the official method, occupies an apparent volume of 0.660 ml. ( $\pm 0.004$  ml.) per gram. This relationship held equally well for a charge of 4 grams of honeydew honey of low moisture content or for one of 8 grams of thin-bodied honey containing 20.7 per cent of moisture.

The projected collaborative study of the two methods for determining dextrin was not carried out, however. Work on projects more directly related to the War effort delayed the organization of the work. Furthermore, a most discerning critique of the two methods, in the light of the 1941 results, made in a letter dated November 16, 1942, by the late General Referee, R. F. Jackson, suggests the empirical character of the official method, and raises serious doubts as to the value of the double alcohol-precipitation method. Jackson wrote:

I am inclined to raise the fundamental question whether the dextrans in honey are completely nonreducing. The nearly 50% occluded reducing sugars (in the crude dextrin precipitated by the alcohol) seems a pretty high degree of occlusion. We are able to ascribe a definite reducing power to the modified starches and even to the modified celluloses. It would seem to me highly probable that the low-molecular-weight dextrans would have reducing properties, and I have thought that this was generally recognized. If this is true, the results by Method I corrected for invert sugar are already too low, but even these corrected results are considerably higher than those by Method II. (Method I was the official, Method II the double precipitation method.)

This raises the further question whether precipitation by alcohol is quantitative. Mr. Ferguson's comments suggest that in some instances it is not. The lower results by Method II, as compared with Method I, can be interpreted in but two ways. Either the crude dextrin precipitate in I is seriously contaminated by noncarbohydrates or the alcoholic precipitation is not quantitative and the double precipitation of II causes the greater loss of dextrin.

The first suggestion, that results by the official method corrected for invert sugar (i.e. corrected for copper-reducing substances) are too low (because the dextrin itself might have reducing power), has recently been fairly well substantiated by the work of J. W. Evans and W. R. Fetzer on dextrans from corn sirup.<sup>1</sup> These chemists also found that as many as nine successive precipitations by alcohol are necessary to obtain a definite corn-sirup dextrin with constant reducing power.

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<sup>1</sup> *Ind. Eng. Chem.*, 35, 439-41 (1943).

Determinations of dextrin and of the "reducing sugar corrections" on the dextrin by the official method, and also after two precipitations by 95-96 per cent alcohol, were made in 1942 by the Associate Referee on several samples of honeydew honey received from different States. Substantial "corrections," based upon copper reduction, were obtained in every case on the dextrans, even after two precipitations; and the size of the correction (calculated as invert sugar) was nearly as large (in proportion to the weight of crude dextrin precipitate) as it was when the dextrin was precipitated only once by the official method. However, the amount of "corrected" dextrin obtained when two alcohol precipitations were made, was only 33-40 per cent of the amount obtained by the official method.

In view of the work on dextrans since the 1941 report, mentioned above, further collaborative work on the double alcoholic precipitation method does not appear to be justified. Instead, efforts should be concentrated upon simplifying and improving the present official method to the point where concordant results can be obtained by different collaborators. It should also be frankly recognized that the official method is an empirical method, and that it does not necessarily yield the true dextrin content of honey or honeydew honey.

The apparent limitations of the official method were recognized by the general referee, and in the letter quoted above he suggested the desirability of working out a method for the determination of pure dextrin. Jackson wrote:

It would, however, be of great value to devise a method which would yield an approximate measure of true dextrin. In a cursory survey of the literature I notice that much attention has been paid to fermentation processes. Most of these, however, have involved the use of selective strains of yeast.

Recently Stark and Somogyi<sup>1</sup> have shown that dextrose can be fermented quantitatively in the presence of maltose at pH 8.4 without effect on the maltose. My guess would be that dextrans would be at least as resistant as maltose. We have used this method with success and have found that levulose is fermented with the dextrose. Possibly this method could be used in the determination of dextrans. It would be necessary to ferment the reducing sugars at the proper pH, separate the yeast by centrifuge, hydrolyze, and determine reducing sugar.

Finally, in view of the work of R. E. Lothrop,<sup>2</sup> and of J. A. Munro,<sup>4</sup> it is suggested that measurement under standardized conditions of the relative viscosity of an aqueous solution of the alcohol precipitate of a honey may provide a method of determining its true dextrin content. The viscosity measurements should be made at standardized total solids content and temperature (*e.g.* 20°C.).

<sup>1</sup> *J. Biol. Chem.*, 142, 579-584 (1942).

<sup>2</sup> *Am. Bee J.*, 79, 130 (1939).

<sup>4</sup> Unpublished thesis "The Viscosity and Thixotropy of Honey," presented at Cornell University, May 1942, in partial fulfillment of the requirement for the Ph.D. degree.

*Free Acid in Honey.*—Some work on the titration of the free acid in honey by R. A. Osborn, 1941 collaborator, and on honey sirups by the Associate Referee indicates that acid is formed at, or near, the neutral point sufficiently rapidly to cause comparatively quick fading of the pink color of the phenolphthalein indicator after progressive additions of alkali. This interference with the establishment of a definite end point of the titration appeared to be accentuated when the temperature of the laboratory was high, and the titration of buckwheat honey seemed to be particularly affected. Presumably, on the alkaline side of the neutral point acidic substances are formed by oxidation of the simple sugars of honey (levulose and dextrose) by the oxygen of the air.<sup>5</sup> If this is assumed to be the case, the true value for the free acid titration would be more nearly determined by titrating only to the point where the faint pink color persists just long enough to make it certain that it was not the result of insufficient mixing; 15 seconds would seem to be sufficient time, but collaborative work should be done to test the point.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the present official method for determining dextrin in honey be recognized as an empirical procedure, and that study be undertaken for the purpose of substituting a Gooch crucible for the filter paper for filtering off the alcohol precipitate.

(2) That the official method for the determination of free acid in honey be studied with a view to making it more definite with respect to the end point of the titration with 0.1 *N* sodium hydroxide by including directions to "titrate until the faint pink color persists for 15 seconds (or some similar short period of time)."

(3) That study of the double alcoholic precipitation method for the determination of dextrin in honey recommended in the 1941 report of this associate referee be discontinued.

(4) That consideration be given, with a view to future work by this Association, to:

(a) The suggestion of the late General Referee regarding the determination of true dextrin in honey by means of precipitation (by alcohol) followed by selective fermentation of occluded sugars;

(b) The possible determination of the dextrin in honey, after its precipitation by alcohol, by determining the viscosity of an aqueous solution of the dextrin under standardized conditions; and

(c) A fundamental study of the characteristic properties of the dextrans of honey and honeydew honey.

<sup>5</sup> Browne and Zerban, "Physical and Chemical Methods of Sugar Analysis," 3rd ed. (1941), 653.

\* For report of Subcommittee D and action by the Association, see *This Journal*, 27, 69 (1944).

## REPORT ON WATERS, BRINE AND SALT

By ANNA E. MIX (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

The Referee has investigated the extent of the use of various methods of reporting results of analyses of samples of water and brine, and the attitude of the workers using these methods. In comparing the reports made by professional workers in America with those made by scientists of European and other foreign countries, it was found that they all recognize the value of the A.O.A.C. tentative method (*Methods of Analysis, A.O.A.C.*, 1940, 543) of reporting results for interpretation of the composition of water and brine samples.

W. D. Collins, in charge of Quality of Water Division, Geological Survey, states:

I would favor the adoption of the method as official for those who wish to use hypothetical combinations in reporting water analyses. It has been recognized that a report of a water analysis in ions does not give all the information that can be obtained from the analytical work. The Geological Survey has made certain computations of properties of natural waters and has made diagrams of analyses, all of which involved assumptions like those made for the computation of hypothetical combinations. These assumptions have been exactly in agreement with the order of combinations of the ions provided for in the A.O.A.C. tentative method, *Book of Methods*, 5th ed., 1940, XXXVII, 81. I believe that the tentative method represents practically universal practice throughout the world in the reporting of water analyses in hypothetical combinations.

R. C. Bardwell, Superintendent of Water Supply, C. & O. Railway Company, states:

The methods for reporting analyses in terms of ions and equivalents per million, as used by the U. S. Geological Survey, and recommended in American Society for Testing Materials Standard D-596-41, are eminently proper for the use of scientists. Even then, they are of no value until the positive and negative ions or equivalents are matched up, or charted as is the practice of Dr. Collins. However, the explanation of constituents found in water and brine as ions or equivalents is merely a cause for bewilderment when reported as such to the general public who have become accustomed to such reports in terms of actual salts with which they are more or less familiar. This may not be entirely scientifically correct, but it is nearly as firmly established as the use of feet and inches and pounds in place of the metric system and for any one dealing with the general public, it is still necessary to continue this practice if he is to be understood at all, or plot up charts as is done by Dr. Collins, which entails considerably extra time not ordinarily available in the usual commercial laboratory.

I have consistently maintained that as long as hypothetical combinations are to be used, which will undoubtedly continue beyond our generation, the most helpful thing that can be done from a scientific standpoint would be to recommend a standard method for making these hypothetical combinations so that the reports will, at least, be uniform and comparable. Analyses published by the American Water Works Association and the American Public Health Association are also included in substantially the same form in the Manual of Recommended Practice of the American Railway Engineering Association, and both of these bodies have voted

to continue this practice. It would therefore not appear out of line for the A.O.A.C. to make it their official standard.

The Referee shares in the views expressed in these communications.

The Associate Referee on Iodides in Salt, Major Tripp, was unable to attend the meeting, and he requested Miss Huntley, who has carried on the associate referee's work since he went into the service, to report the results obtained and the various phases connected with the determination of iodide in salt. Progress has been made by the associate referee in the study of methods for this determination and also by the Referee, associated with R. A. Osborn and L. L. Ramsey (*This Journal*, 26, 440).

The associate referee believes that the modification of the Elmslie-Caldwell method used in his work and the collaborative results obtained warrant the adoption of this method. The method developed by Osborn, Mix, and Ramsey has not had sufficient collaborative study. However, the results obtained appear to be only slightly lower than those found by the associate referee using the modified Elmslie-Caldwell method.

The Referee conducted a few experiments on the determination of fluorine in salt, and the results look promising, but it is considered that a report at this time would be of little value. The Referee had no opportunity to work on methods for the determination of boron in water.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the tentative method of reporting results on waters and brine (*Methods of Analysis*, A.O.A.C., 1940, 543, 81) and the table of combining weights and their reciprocals (*Ibid.*, 554, 82) be made official (first action). These figures were calculated from the International Atomic Weights of 1939, but no change was noted in the 1943 table.

(2) That study of methods for the determination of boron in waters be continued.

(3) That the tentative method for preparation of sample of salt (*Ibid.*, 549, 113), and the tentative method for the determination of sulfate in salt, *Ibid.*, 550, 118), be further studied.

(4) That the method for the determination of fluorides in water (*Ibid.*, 529, 22–25), corrected and reprinted to show changes made in 1940–41 (*This Journal*, 25, 101) be made official (final action).

(5) That methods for the determination of fluorine in salt be studied.

(6) That methods for sampling iodized salt be further studied.

(7) That tentative methods I and II for the determination of iodides in salt (*Methods of Analysis*, A.O.A.C., 1940, 547 and 548, 100–105) be dropped because newer methods are more rapid and produce accurate results.

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\* For report of Subcommittee D and action by the Association, see *This Journal*, 27, 70.

(8) That methods for the determination of iodides in salt (*Ibid.*, 368, 58; *This Journal*, 26, 440; *Ind. Eng. Chem., Anal. Ed.*, 5, 368) be studied collaboratively.

(9) That the Elmslie-Caldwell procedure for the determination of iodine in mineral mixed feeds (*Methods of Analysis*, A.O.A.C., 1940, 368, 58), as modified by the Associate Referee on Salt (*This Journal*, 27, 110) be made official (first action) for the determination of iodine in salt.

## REPORT ON IODIDES IN STABILIZED IODIZED SALT\*

By LAURA M. HUNTLEY, *Associate Referee*, and J. T. TRIPP†  
(Bureau of Laboratories, Michigan Department of  
Health, Lansing, Mich.)

The American Public Health Association in January, 1942, presented the following resolution:

*Resolved*, That all salt for table use of human beings and salt used for feeding to domestic animals in the United States should contain one hundredth of one per cent of Potassium Iodide or its equivalent, viz., forty-five milligrams of Potassium Iodide to each pound of salt, provided that an effective stabilizer be used.

This resolution was the result of collaborative work and recommendations previously made by the American Public Health Association, Committee on Study of Endemic Goitre; National Research Council, Committee on Food and Nutrition; and the American Medical Association, Council on Food and Nutrition.

The reduction in iodine content, coupled with the use of various stabilizers, raised the question of the suitability of the existing methods for analyzing such iodized salt. At that time it was not known whether the tentative method of the A.O.A.C.<sup>1</sup> for the determination of iodides in brine would give accurate results on these preparations. Therefore, this study was initiated to evaluate analytical procedures for the determination of iodides in stabilized iodized salt.

The appointment of the A.O.A.C. Associate Referee on Salt to the American Public Health Association Committee on Standard Methods prevented duplication of work. Further coordination was secured by the appointment of members of the American Medical Association and National Research Council to the American Public Health Association Committee on Endemic Goitre. Members of the salt producing industry were invited to present the technical difficulties incident to the adoption of a reduction in potassium iodide content, and to suggest the technical needs

\* Submitted in partial fulfillment for the Degree of Master of Science, Department of Bacteriology, Michigan State College, 1944.

† Former Associate Referee.

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1940, 547, 102.



of the industry regarding control methods. The Salt Producers Association has given support to the work of the Associate Referee through its Committee on Standard Practices.

The work to date has been divided into two phases. Phase I is a collaborative study designed to evaluate the methods routinely used by control analysts. Each collaborator was asked to use a method of his own choice in analyzing the samples submitted to him. Phase II also is a collaborative study designed to evaluate further the two methods (both of which gave satisfactory results) most frequently selected by the collaborators in Phase I of the study. Each analyst was requested to use the two specified methods in analyzing the samples submitted to him in the second phase of the study.

### PHASE I

*Samples used.*—Each of nine salt producing companies located in various areas throughout the United States and employing different stabilizers collected 24 cartons of iodized salt from their production lines.

*Preparation of samples.*—The contents of ten of the cartons from each company were pooled, assigned a sample number, and thoroughly mixed. Aliquots of the salt were accurately weighed, and 20 liters of a 20 per cent solution was prepared from each of the nine salt samples submitted. Sufficient acid (concentrated sulfuric or concentrated hydrochloric, depending on the nature of the stabilizers) was added to dissolve the stabilizers. The quantities of concentrated acids added were as follows:

<i>Sample No.</i>	<i>Stabilizers</i>	<i>Acid added/ liter solution ml.</i>
1	MgCO <sub>3</sub>	0.75 H <sub>2</sub> SO <sub>4</sub>
2	MgCO <sub>3</sub>	0.50 H <sub>2</sub> SO <sub>4</sub>
3	MgCO <sub>3</sub>	1.00 H <sub>2</sub> SO <sub>4</sub>
4	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> NaHCO <sub>3</sub> Dextrose	0.89 HCl
5	MgCO <sub>3</sub>	0.75 H <sub>2</sub> SO <sub>4</sub>
6	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	2.00 HCl
7	MgCO <sub>3</sub> CaO Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	0.80 H <sub>2</sub> SO <sub>4</sub>
8	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> NaHCO <sub>3</sub>	2.00 HCl
9	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> NaHCO <sub>3</sub>	2.75 HCl

The solutions were sent to the collaborators in amber glass bottles, sealed with "celoseal" caps, and labeled to show sample number, concentration of salt, and kind and amount of acid added.

*Collaborating analysts.*—Aliquots of all nine samples were submitted to the following collaborating analysts, each of whom was asked to use a method of his choice in analyzing for per cent iodine.

<i>Organization</i>	<i>Analyst</i>
American Salt Company, Kansas City, Mo.	Kansas City Testing Laboratory
Barton Salt Company, Hutchinson, Kan.	R. S. Humphreys
Carey Salt Company, Hutchinson, Kan.	L. A. Enberg
*Colonial Salt Company, Akron, Ohio	L. C. Judy
*Diamond Crystal Salt Company, St. Clair, Mich.	H. W. Diamond
*Hardy (Manistee) Salt Company, Manistee, Mich.	C. G. Lindstrom
*International Salt Company, Watkins Glen, N. Y.	A. Marciniak
Jefferson Island Salt Company, Jefferson Island, La.	J. Grant-Mackay
Leslie Salt Company, Newark, Calif.	D. S. See
Michigan Department of Health, Lansing, Mich.	Laura Huntley
*Morton Salt Company, Chicago, Ill.	C. H. Martin
Myles Salt Company, Weeks, La.	Staff Chemists
*Ohio Salt Company, Rittman, Ohio	T. R. Rader
*Union Salt Company, Cleveland, Ohio	The Textor Laboratories
U. S. Department of Agriculture, Washington, D. C.	R. A. Osborn
Wisconsin Department of Agriculture, Madison, Wis.	E. LeMense
*Worcester Salt Company, Silver Springs, N. Y.	F. D. Everett

\* Furnished salt samples, as did also the Watkins Salt Company, Watkins Glen, N. Y.

*Methods employed.*—The following methods of analysis were selected by the collaborating analysts:

- I. Elmslie-Caldwell<sup>2</sup>
- II. Sadusk-Ball<sup>3</sup>
- III. Potassium cyanide (original reference not available)
- IV. Tentative method for brine, A.O.A.C.<sup>4</sup>
- V. Woodward<sup>5</sup>
- VI. Fresenius<sup>6</sup>
- VII. Viebock and Breckner<sup>6,7</sup> method modified by New York Station of Food and Drug Administration.

## RESULTS AND CONCLUSIONS

The results submitted by the 17 collaborating analysts are presented in Table 1.

<sup>2</sup> *Methods of Analysis*, A.O.A.C., 1940, 388, 58.

<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 5, 386 (1933).

<sup>4</sup> *Mich. Dept. of Agr. Bull.*, 38, 14 (1924-5); *Am. J. Pub. Health*, 19, 991 (1929).

<sup>5</sup> Furman, "Scott's Standard Methods of Analysis," Vol. 1, 454 (1939).

<sup>6</sup> *Pharm. Monats.*, 10, 191 (1929).

<sup>7</sup> *This Journal*, 17, 486 (1934).

TABLE 1.—Iodine ( $I_2$ ) content of iodized salts determined by collaborating analysts employing methods of their own choice

SAMPLE NO.	LABELED CONSTITUENTS	METHOD I		METHOD II		METHOD III		METHOD IV		METHOD V		METHOD VI		METHOD VII	
		ANALYST	$I_2$ per cent	ANALYST	$I_2$ per cent	ANALYST	$I_2$ per cent	ANALYST	$I_2$ per cent	ANALYST	$I_2$ per cent	ANALYST	$I_2$ per cent	ANALYST	$I_2$ per cent
1	$MgCO_3$ KI	A	.0107	B	.0105	D	.0107	F	.0109	H	.0102	I	.0118	Q	.0107
		C	.0109	C	.0109	K	.0121	M	.0109	J	.0107				
		E	.0105	G	.0116			N	.0118						
2	$MgCO_3$ KI	I	.0117	O	.0107										
		L	.0108												
		O	.0106												
3	$MgCO_3$ KI	Median	.0108		.0108		.0114		.0109		.0104		.0118		.0107
		Mean	.0109		.0109		.0114		.0112		.0104		.0118		.0107
		A	.0174	B	.0175	D	.0192	F	.0174	H	.0179	I	.0180	Q	.0188
4	$NaHCO_3$ Dextrose $Ca_3(PO_4)_2$ KI	C	.0176	C	.0176	K	.0178	M	.0177	J	.0176				
		E	.0175	G	.0177			N	.0177						
		I	.0185	O	.0174				.0194						
5	$MgCO_3$ KI	L	.0172												
		O	.0174		.0176		.0182		.0177		.0178		.0180		.0188
		Median	.0175		.0176		.0182		.0182		.0178		.0180		.0188
6	$MgCO_3$ KI	A	.0084	B	.0082	D	.0092	F	.0088	H	.0084	I	.0098	Q	.0096
		C	.0086	C	.0087	K	.0097	M	.0089	J	.0092				
		E	.0080	G	.0087			N	.0106						
7	$MgCO_3$ KI	I	.0088	O	.0083										
		L	.0084												
		O	.0084												
8	$MgCO_3$ KI	Median	.0084		.0087		.0094		.0089		.0088		.0098		.0096
		Mean	.0086		.0085		.0094		.0094		.0088		.0098		.0096
		A	.0153	B	.0149	D	.0163	F	.0155	H	.0164	I	.0162	Q	.0163
9	$MgCO_3$ KI	C	.0155	C	.0156	K	.0186	M	.0158	J	.0163				
		E	.0160	G	.0153			N	.0162						
		I	.0164	O	.0157										
10	$MgCO_3$ KI	L	.0153		.0153										
		O	.0162												
		Median	.0154		.0153		.0177		.0153		.0158		.0162		.0163
11	$MgCO_3$ KI	Mean	.0156		.0154		.0177		.0158		.0158		.0162		.0163
		A	.0159	B	.0151	D	.0176	F	.0163	H	.0164	I	.0174	Q	.0170
		C	.0161	C	.0161	K	.0164	M	.0163	J	.0153				
12	$MgCO_3$ KI	E	.0155	G	.0146			N	.0183						
		I	.0176	O	.0162										
		L	.0159		.0158										
13	$MgCO_3$ KI	O	.0158												
		Median	.0150		.0158		.0170		.0164		.0158		.0174		.0170
		Mean	.0161		.0156		.0170		.0170		.0158		.0174		.0170

TABLE 1.—Continued

SAMPLE NO.	LABELED CONSTITUENTS	METHOD I		METHOD II		METHOD III		METHOD IV		METHOD V		METHOD VI		METHOD VII	
		ANALYST	I <sub>s</sub>	ANALYST	I <sub>s</sub>	ANALYST	I <sub>s</sub>	ANALYST	I <sub>s</sub>	ANALYST	I <sub>s</sub>	ANALYST	I <sub>s</sub>	ANALYST	I <sub>s</sub>
6	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> KI	A	.0122	B	.0116	D	.0138	F	.0125	H	.0112	I	.0127	Q	.0124
		C	.0123	C	.0123	K	.0149	M	.0135	J	.0115				
		E	.0123	G	.0133			N	.0141						
7	MgCO <sub>3</sub> CaO Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> KI	I	.0128	O	.0123										
		L	.0123	P	.0120										
		O	.0120												
8	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> NaHCO <sub>3</sub> KI	Median	.0123		.0123		.0144		.0135		.0114		.0127		.0124
		Mean	.0124				.0144		.0134		.0114		.0127		.0124
		A	.0088	B	.0083	D	.0275	F	.0096	H	.0085	I	.0086	Q	.0086
9	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> NaHCO <sub>3</sub> KI	C	.0088	C	.0089	K	.0098	M	.0091	J	.0099				
		E	.0085	G	.0086			N	.0086						
		I	.0093	O	.0090										
9	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> NaHCO <sub>3</sub> KI	L	.0086	P	.0084										
		O	.0087												
		Median	.0088		.0086		.0186		.0091		.0092		.0096		.0086
8	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> NaHCO <sub>3</sub> KI	Mean	.0088		.0086		.0186		.0091		.0092		.0096		.0086
		A	.0175	B	.0164	D	.0206	F	.0170	H	.0153	I	.0183	Q	.0178
		C	.0177	C	.0177	K	.0205	M	.0180	J	.0176				
9	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> NaHCO <sub>3</sub> KI	E	.0165	G	.0164			N	.0183						
		I	.0185	O	.0170										
		L	.0173	P	.0170										
9	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> NaHCO <sub>3</sub> KI	O	.0146												
		Median	.0174		.0170		.0206		.0180		.0164		.0183		.0178
		Mean	.0170		.0169		.0206		.0178		.0164		.0183		.0178
9	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> NaHCO <sub>3</sub> KI	A	.0203	B	.0194	D	.0322	F	.0207	H	.0186	I	.0229	Q	.0206
		C	.0204	C	.0206	K	.0300	M	.0207	J	.0214				
		E	.0200	G	.0206			N	.0211						
9	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> NaHCO <sub>3</sub> KI	I	.0226	O	.0208										
		L	.0200	P	.0200										
		O	.0205												
9	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> NaHCO <sub>3</sub> KI	Median	.0204		.0200		.0261		.0207		.0200		.0229		.0206
		Mean	.0206		.0198		.0261		.0208		.0200		.0229		.0206

The two most popular methods, as evidenced by the number of analysts who selected them, were the Elmslie-Caldwell<sup>2</sup> (Method I) and the Sadusk-Ball<sup>3</sup> (Method II). Both procedures gave satisfactory results as judged by their distribution about the mean values. The other five methods were less frequently used, and while the results in some cases were apparently erroneous there are not sufficient data to prove or disprove their value as analytical procedures. By the same token there are not sufficient data to justify the acceptance of either Method I or Method II as completely satisfactory procedures. These data only indicate that Methods I and II were used most frequently by this collaborating group of analysts and that the results obtained showed deviations that were within reasonable limits.

## PHASE II

*Source of samples.*—The following salt producing companies, located in various areas and employing different stabilizers, collected a number of cartons of iodized salt from their production lines at each of several specified time intervals during two consecutive days.

Hardy (Manistee) Salt Company  
Jefferson Island Salt Company  
Ohio Salt Company  
Watkins Salt Company

*Preparation of samples.*—Manufacturers code letters A, B, C, and D were assigned to the samples, and to each of the code letters was added the serial number given by the individual producer to denote order of collection (e.g., A-1, A-2, A-3, etc.). Each sample was prepared by pooling and thoroughly mixing the contents of four cartons bearing the same code letter and number (e.g., A-1). Aliquots of salt were accurately weighed, and 6 liters of a 20 per cent solution was prepared from each of the samples collected at specified intervals by the four companies; 1 ml. of concentrated sulfuric acid per liter of solution was added to each sample to dissolve the stabilizers. The solutions were placed in amber glass bottles, sealed with "celoseal" caps, and labeled to show sample number, concentration of salt, and amount of sulfuric acid added.

*Collaborating analysts.*—Six collaborating analysts were sent Samples A-1 to A-9 and B-1 to B-9, and six other analysts were sent Samples C-1 to C-9 and D-1 to D-6. However one analyst of the second group failed to submit results, and another analyst, because of technical difficulties, was unable to submit an acceptable report. It was unfortunate that both analysts had received the C and D series of samples. The names of the 10 collaborating analysts submitting acceptable results follow:

Barton Salt Company, Hutchinson, Kan.  
Carey Salt Company, Hutchinson, Kan.  
Colonial Salt Company, Akron, Ohio

R. S. Humphreys  
L. A. Enberg  
L. C. Judy

Diamond Crystal Salt Company, St. Clair, Mich.

Hardy (Manistee) Salt Company, Manistee, Mich.

International Salt Company, Watkins Glen, N. Y.

Jefferson Island Salt Company, Jefferson, Island, La.

Michigan Department of Health, Lansing, Mich.

Morton Salt Company, Chicago, Ill.

Ohio Salt Company, Rittman, Ohio

F. G. Miller and

J. E. LaPorte

C. G. Lindstrom

A. Marciniak

J. Grant-Mackay

Laura Huntley

C. H. Martin

T. R. Rader

*Methods employed.*—The collaborating analysts were asked to analyze the samples for per cent iodine by both the Elmslie-Caldwell<sup>2</sup> and Sadusk-Ball<sup>3</sup> procedures, which in Phase I of the study gave satisfactory results, and were most frequently selected by the collaborators. The procedures are as follows:

#### IODIDE IN SALT

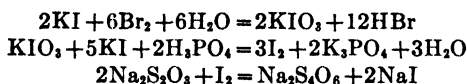
##### *Method I.—Elmslie-Caldwell<sup>2</sup>*

##### REAGENTS

(a) *Sodium thiosulfate solution.*—0.005 *N*. Dissolve 12.4 grams of reagent-grade  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in freshly distilled water and dilute to 1 liter, giving an approximately 0.05 *N* stock solution. Prepare 0.005 *N* solutions by dilution of this stock solution with freshly distilled water.

(b) *Standard potassium iodide solution.*—Prepare a solution containing exactly 0.1308 gram of reagent-grade KI (0.1000 g  $\text{I}_2$ ) per 1 liter of freshly distilled water.

##### REACTIONS



##### DETERMINATION

In a flask place 25 ml. aliquots of a 20% solution of iodized salt, add 5 grams of  $\text{Na}_2\text{CO}_3$ , cover flask with watch-glass, and boil gently for 10 minutes. Transfer contents of flask to an 18 cm. filter paper and wash with boiling water, catching filtrate and washings in 500 ml. Erlenmeyer flask (solution should total about 300 ml.). Neutralize to methyl orange with 85%  $\text{H}_3\text{PO}_4$ , and add 1 ml. in excess.

Add excess of Br water and boil solution gently until colorless, and then 5 minutes longer. Add a few crystals of salicylic acid and cool to approximately 20°. Add 1 ml. of 85%  $\text{H}_3\text{PO}_4$  and approximately 0.5 gram of KI and titrate the I with 0.005 *N*  $\text{Na}_2\text{S}_2\text{O}_3$  in the usual way, using starch solution as indicator.

Standardize the  $\text{Na}_2\text{S}_2\text{O}_3$  solution by measuring into a 500 ml. Erlenmeyer flask exactly 25 ml. of the standard KI solution, 300 ml. of water and 5 grams of  $\text{Na}_2\text{CO}_3$ , neutralizing, and proceeding as directed above, beginning "Add excess of Br water . . ."

It is advisable to standardize the  $\text{Na}_2\text{S}_2\text{O}_3$  solution same day determinations are conducted.

##### *Method II.—Sadusk-Ball<sup>3</sup>*

##### REAGENTS

(a) *Sulfuric acid.*—2 *N*. 1 volume of concentrated  $\text{H}_2\text{SO}_4$  plus 17 volumes of freshly distilled water.

TABLE 2.—*Iodine (I<sub>2</sub>) content of iodized salts determined by collaborating analysis employing Elmslie-Caldwell (I) and Sadusk-Ball (II) methods of analysis*

SAMPLE NO.	TIME OF COLLECTION	ANALYST 1		ANALYST 2		ANALYST 3		ANALYST 4		ANALYST 5		ANALYST 6		MEAN		RANGE		MEDIAN	
		I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
A. Samples A-1 to A-9, labeled as containing 0.010% KI and 0.8% MgCO <sub>3</sub> ; Samples B-1 to B-9 labeled as containing 0.010% KI, 0.5% MgCO <sub>3</sub> , 1.0% CaO, and 1.0% Na <sub>2</sub> SO <sub>4</sub> .																			
A-1	First day 9 A.M.	.0100	.0104	.0103	.0102	.0104	.0101	.0106	.0107	.0097	.0097	.0103	.0101	.0102	.0102	.0097-.0106	.0097-.0107	.0103	.0102
A-2	First day 10 A.M.	.0110	.0118	.0112	.0100	.0112	.0110	.0116	.0115	.0113	.0109	.0112	.0109	.0112	.0112	.0110-.0116	.0100-.0118	.0112	.0113
A-3	First day 12 noon	.0080	.0084	.0082	.0084	.0083	.0086	.0083	.0084	.0079	.0079	.0080	.0083	.0081	.0083	.0079-.0083	.0079-.0086	.0081	.0084
A-4	First day 2 P.M.	.0138	.0138	.0144	.0143	.0143	.0142	.0142	.0142	.0143	.0140	.0143	.0142	.0142	.0141	.0138-.0144	.0138-.0143	.0143	.0142
A-5	Second day 9 A.M.	.0181	.0182	.0184	.0183	.0183	.0179	.0186	.0187	.0183	.0180	.0184	.0179	.0184	.0182	.0181-.0186	.0179-.0187	.0184	.0181
A-6	Second day 10 A.M.	.0141	.0145	.0146	.0150	.0148	.0148	.0137	.0137	.0144	.0143	.0146	.0143	.0144	.0144	.0137-.0148	.0137-.0150	.0145	.0144
A-7	Second day 12 noon	.0122	.0125	.0124	.0124	.0120	.0121	.0131	.0132	.0126	.0122	.0124	.0123	.0124	.0124	.0120-.0131	.0121-.0132	.0124	.0124
A-8	Second day 1 P.M.	.0142	.0143	.0152	.0157	.0145	.0141	.0145	.0144	.0146	.0146	.0145	.0145	.0146	.0146	.0142-.0152	.0141-.0157	.0145	.0144
A-9	Second day 2 P.M.	.0114	.0118	.0138	.0136	.0134	.0135	.0135	.0136	.0135	.0133	.0137	.0132	.0132	.0132	.0114-.0138	.0118-.0136	.0135	.0134
B-1	First day 9 A.M.	.0078	.0073	.0073	.0073	.0075	.0076	.0073	.0074	.0077	.0075	.0077	.0074	.0076	.0074	.0073-.0078	.0073-.0076	.0076	.0074
B-2	First day 10 A.M.	.0067	.0068	.0070	.0067	.0070	.0068	.0068	.0068	.0070	.0067	.0070	.0069	.0069	.0068	.0067-.0070	.0067-.0069	.0070	.0068
B-3	First day 12 noon	.0067	.0073	.0075	.0074	.0077	.0075	.0074	.0075	.0076	.0073	.0074	.0074	.0074	.0074	.0067-.0077	.0073-.0075	.0074	.0074
B-4	First day 2 P.M.	.0068	.0070	.0074	.0075	.0075	.0074	.0073	.0074	.0073	.0071	.0074	.0074	.0073	.0073	.0068-.0075	.0070-.0075	.0074	.0074
B-5	Second day 9 A.M.	.0067	.0067	.0068	.0066	.0071	.0068	.0065	.0064	.0068	.0065	.0068	.0066	.0068	.0066	.0065-.0071	.0064-.0068	.0068	.0066
B-6	Second day 10 A.M.	.0068	.0070	.0072	.0070	.0072	.0074	.0070	.0071	.0072	.0070	.0072	.0071	.0071	.0071	.0068-.0072	.0070-.0074	.0072	.0070
B-7	Second day 12 noon	.0072	.0073	.0075	.0075	.0076	.0076	.0073	.0073	.0077	.0072	.0075	.0078	.0075	.0074	.0072-.0077	.0072-.0078	.0075	.0074
B-8	Second day 1 P.M.	.0067	.0076	.0072	.0071	.0070	.0068	.0072	.0072	.0067	.0071	.0069	.0070	.0070	.0071	.0067-.0072	.0068-.0076	.0070	.0071
B-9	Second day 2 P.M.	.0063	.0066	.0068	.0066	.0064	.0064	.0066	.0066	.0068	.0064	.0066	.0066	.0066	.0065	.0063-.0068	.0064-.0066	.0066	.0066

TABLE 2.—Continued

SAM- PLI- NO.	TIME OF COLLECTION	ANALYST 7		ANALYST 8		ANALYST 9		ANALYST 10		MEAN		RANGE		MEDIAN	
		I per cent	II per cent	I per cent	II per cent	I per cent	II per cent	I per cent	II per cent	I per cent	II per cent	I per cent	II per cent	I per cent	II per cent
B. Samples C-1 to C-9, labeled as containing 0.010% KI, and Ca(CO <sub>3</sub> )+MgCO <sub>3</sub> < 1%; Samples D-1 to D-6, labeled as containing 0.020% KI, and 1% MgCO <sub>3</sub> .															
C-1	First day 9 A.M.	.0063	.0062	.0058	.0058	.0062	.0062	.0057	.0060	.0060	.0060	.0057-. 0063	.0058-. 0062	.0060	.0061
C-2	First day 10 A.M.	.0054	.0054	.0053	.0053	.0058	.0051	.0051	.0053	.0054	.0053	.0051-. 0058	.0051-. 0054	.0054	.0053
C-3	First day 12 noon	.0044	.0044	.0045	.0045	.0036	.0039	.0042	.0045	.0042	.0043	.0036-. 0045	.0039-. 0045	.0043	.0044
C-4	First day 2 P.M.	.0036	.0034	.0036	.0036	.0036	.0036	.0036	.0037	.0036	.0036	.0036-. 0036	.0034-. 0037	.0036	.0036
C-5	Second day 9 A.M.	.0063	.0063	.0061	.0061	.0062	.0066	.0061	.0062	.0062	.0063	.0061-. 0063	.0061-. 0066	.0062	.0062
C-6	Second day 10 A.M.	.0079	.0080	.0076	.0078	.0079	.0083	.0078	.0080	.0078	.0080	.0076-. 0079	.0078-. 0083	.0078	.0080
C-7	Second day 12 noon	.0063	.0063	.0059	.0059	.0060	.0062	.0059	.0061	.0060	.0061	.0059-. 0063	.0059-. 0063	.0060	.0062
C-8	Second day 1 P.M.	.0057	.0056	.0054	.0054	.0057	.0055	.0055	.0057	.0056	.0056	.0054-. 0057	.0054-. 0057	.0056	.0056
C-9	Second day 2 P.M.	.0062	.0063	.0059	.0059	.0063	.0063	.0060	.0062	.0061	.0062	.0059-. 0063	.0059-. 0063	.0061	.0062
D-1	First day 11:30 A.M.	.0070	.0069	.0067	.0068	.0075	.0075	.0068	.0070	.0070	.0071	.0067-. 0075	.0068-. 0075	.0069	.0070
D-2	First day 1 P.M.	.0087	.0086	.0083	.0083	.0086	.0089	.0085	.0088	.0085	.0086	.0083-. 0087	.0083-. 0089	.0086	.0087
D-3	First day 1:30 P.M.	.0074	.0073	.0073	.0073	.0076	.0079	.0073	.0076	.0074	.0075	.0073-. 0076	.0073-. 0079	.0074	.0074
D-4	First day 2:30 P.M.	.0071	.0071	.0067	.0068	.0070	.0072	.0068	.0069	.0069	.0070	.0067-. 0071	.0068-. 0072	.0069	.0070
D-5	First day 3:30 P.M.	.0083	.0083	.0080	.0080	.0081	.0079	.0079	.0082	.0081	.0081	.0079-. 0083	.0079-. 0083	.0080	.0081
D-6	First day 4 P.M.	.0076	.0075	.0073	.0074	.0077	.0079	.0074	.0074	.0075	.0076	.0073-. 0077	.0074-. 0079	.0075	.0074



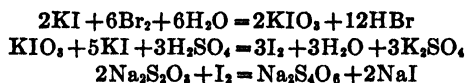
(b) *Bromine vapor*.—Blow air (washed with water and dried by  $\text{CaCl}_2$ ) through a gas-washing tube containing C.P. bromine. Deliver vapors just above the liquid in the flask.

(c) *Potassium iodide solution*.—10%. 10 grams of reagent-grade KI/100 ml. (freshly prepared).

(d) *Sodium thiosulfate solution*.—0.005 *N*. Dissolve 1.24 grams of reagent-grade  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  and 3.8 grams of crystallized borax ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) in freshly distilled water and dilute to 1 liter. Standardize the solution immediately before use by titration against standard  $\text{KIO}_3$  solution.

(e) *Standard potassium iodate solution*.—0.005 *N*. Prepare a stock solution containing exactly 3.567 grams of reagent-grade  $\text{KIO}_3$  per liter, giving a 0.1 *N* solution. Further dilute the stock solution to give a 0.005 *N* reagent.

#### REACTIONS



#### DETERMINATION

Transfer a 5 gram portion of the salt and 100 ml. of freshly distilled water to a 200 ml. Erlenmeyer flask and mix the contents of the vessel until solution of the sodium chloride has taken place. Add methyl orange indicator and introduce 2 *N*  $\text{H}_2\text{SO}_4$  drop by drop until a pink color results which persists on standing. After the addition of 2 ml. of 2 *N*  $\text{H}_2\text{SO}_4$  and Br vapor, add a few glass beads and boil the solution until the yellow color due to bromine disappears and then for 2 minutes longer. Upon cooling, wash down the sides of the flask with approximately the amount of water lost by boiling. Add 2 ml. of the 10% KI solution (freshly prepared) gently swirl the contents of the flask, and titrate liberated iodine immediately with 0.005 *N* thiosulfate, adding 2 ml. of starch indicator near the end point.

#### RESULTS AND CONCLUSIONS

The results of the 10 collaborating analysts are given in Table 2. These data show that there was close agreement between the results submitted by individual analysts and that the Elmslie-Caldwell and Sadusk-Ball methods are equally suitable as analytical procedures for the determination of iodine in stabilized iodized salt.

#### RECOVERY EXPERIMENTS

In order to evaluate further the two methods for the determination of iodine in stabilized iodized salt, recovery experiments were performed by the Associate Referee. In both procedures reagent-grade potassium iodate (Bakers analyzed) was used to standardize the 0.005 *N* sodium thiosulfate solution. The iodine contents of the iodized salt solution and of the stock potassium iodide solution were determined by a series of six analyses on each, and mean values were used in the calculation of the recoveries. The results of these recovery series are given in Table 3. These data are in agreement with those previously presented, and they further indicate that both methods are sufficiently accurate for the determination of iodine in stabilized iodized salt.

TABLE 3.—*Recoveries of iodine added as KI to 25 ml. of a 20% iodized salt solution*

TRIAL NUMBER	I <sub>2</sub> CONTENT OF IODIZED SALT SOLUTION	I <sub>2</sub> ADDED AS KI	TOTAL I <sub>2</sub>	DETERMINED I <sub>2</sub>	RECOVERY
A. By Elmslie-Caldwell method					
	mg.	mg.	mg.	mg.	per cent
1	.6714	.2236	.8950	.8979	100.32
2	.6714	.2236	.8950	.8817	98.51
3	.6714	.4472	1.1186	1.1122	99.43
4	.6714	.4472	1.1186	1.1214	100.25
5	.6714	.8943	1.5657	1.5948	101.86
6	.6714	.8943	1.5657	1.5927	101.72
B. By Sadusk-Ball method					
1	.6973	.2236	.9209	.9194	99.84
2	.6973	.2236	.9209	.9206	99.97
3	.6973	.4472	1.1445	1.1468	100.21
4	.6973	.4472	1.1445	1.1427	99.85
5	.6973	.8943	1.5916	Lost	—
6	.6973	.8943	1.5916	1.5974	100.36

## SUMMARY AND RECOMMENDATIONS

In a collaborative study it was found that the Elmslie-Caldwell and Sadusk-Ball methods for the determination of iodine in stabilized iodized salt were most frequently employed for routine analyses in the laboratories of salt producers. The results of the collaborative study and the recovery experiments performed by the Associate Referee show that both methods are sufficiently accurate and reproducible for the purpose designated.

On the basis of these findings, the Associate Referee makes the following recommendations.\*

(1) That the Elmslie-Caldwell method be considered for adoption as an official method for the determination of iodine in stabilized iodized salt.

(2) That the Sadusk-Ball method be considered for adoption as a tentative method for the determination of iodine in stabilized iodized salt.

## ACKNOWLEDGMENTS

The authors wish to express their appreciation to all collaborating analysts.

\* For report of Subcommittee D and action by the Association, see *This Journal*, 27, 71 (1944).

## REPORT ON ADULTERATION OF CONDENSED MILK PRODUCTS

By P. B. CURTIS (Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Associate Referee*

At the 1937 meeting of this Association a qualitative method for the detection of starch in condensed milk products was presented. In 1938 the method was studied collaboratively, and the results of that work were published in *This Journal*, 22, 676. In 1939, the method with slight modifications was studied by 13 collaborators, and the results of that study were published in *This Journal*, 23, 656. As a result of the two years of collaborative work and the favorable comments of the collaborators the method was adopted as tentative in 1939 and was published in *Methods of Analysis, A.O.A.C.*, 1940, 361, 34. In 1941 the tentative method was adopted as official (first action).

Since collaborative study has shown this method to be reliable and to have practical value in detecting starch qualitatively in condensed and dried milk products and since no criticism of the method has been received during the last four years, it is recommended\* that the method be made official (final action), and that study be discontinued.

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REPORT ON AMMONIACAL UREA AND NITROGEN SALTS

By W. B. GRIEM (State Department of Agriculture, Madison Wis.), *Associate Referee*

No new collaborative work was undertaken by the Associate Referee. Collaborative work carried out in 1940 (*This Journal*, 24, 867) led to the adoption of a tentative method for the determination of ammoniacal and urea nitrogen in feeds. The work at that time was undertaken as an emergency study project because urea had been proved to be a partial substitute for protein in the ration of the ruminant.

Collaborative work was again carried out in 1941 (*Ibid.*, 25, 874). This led to the establishment of the method as official (first action) after several minor changes had been incorporated. Agreement in collaborative results was excellent both times. No information has been uncovered, which in the Associate Referee's judgment would improve the method, and he considers that the work can be discontinued at least for the present.

## RECOMMENDATIONS\*

It is recommended—

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\* For report of Subcommittee A and action by the Association, See *This Journal*, 27, 45 (1944).

(1) That the method, official (first action), for the determination of ammoniacal and urea nitrogen (*This Journal*, 24, 79), as amended (*Ibid.* 25, 93) be made official (final action).

(2) That no associate referee on this subject be appointed at this time.

## REPORT ON YEAST ACTIVITY

By H. J. WITTEVEEN (Department of Agriculture, Dairy  
& Food, St. Paul, Minn.), *Associate Referee*

The study of yeast activity has for its purpose the development of a method which may be used to determine the quality of a class of feed-stuffs known as yeast cultures. On the basis of such a method suitable standards for this class of feeds can be established. While diversity of opinion exists as to the merits of using fermented feeds, it is self-evident that as long as there is a sufficient demand for yeast cultures to make production of such materials a successful business enterprise, standards of quality are desirable. Proper standards would be helpful in determining whether or not the products will serve the purpose for which they are intended.

At the present time it seems that methods that best measure yeast activity are those that involve the production of carbon dioxide gas. Methods that measure yeast activity by the amount of carbon dioxide produced vary in the nature of the media and the type of apparatus used. War conditions place a very definite limitation on the equipment that may be procured. In addition simplicity of design and operation is desirable in order to make the methods acceptable to the largest number of operators.

The apparatus used in this study consisted of two 500 ml. wide-mouthed bottles and a 50 ml. graduated cylinder, all connected by glass tubing. The carbon dioxide gas formed from the sample in the one bottle passed into the second bottle, which was filled with a saturated solution of common salt. The displaced salt solution was received in the graduated cylinder. The entire apparatus was placed in a hot air oven at 35°C. A 3-gram sample was used and to it was added 5 grams of bacto malt extract, 75 ml. of water, and 25 ml. of buffer solution of the following composition: 100 grams of potassium citrate, 20 grams of citric acid, 20 grams of mono-ammonium phosphate in 1000 ml of water.

During the test the bottle containing the sample was shaken every 15 minutes, and readings were taken every 30 minutes for 6 hours.

After preliminary tests had been made, an outline of the method and a sample to be tested were submitted to the collaborators for trial. The

collaborators who so generously participated in this work are the following:

Jack Stebner, State Laboratory Department, Bismarck, North Dakota.

Guy G. Frary, State Chemical Laboratory, Vermillion, South Dakota.

A. T. Perkins, Department of Chemistry, Kansas State College, Manhattan, Kansas.

L. L. Boughton, School of Pharmacy, University of Kansas, Lawrence, Kansas.

M. H. Givens, Northwestern Yeast Co., Chicago, Ill.

Tests were made each week for a period of 4 months. Results obtained were not in close accord, however. Workers reported results which agreed more closely with themselves than with each other. One or two collaborators reported negative results. A study of these results seemed to indicate that either the method was faulty in some respect or that the sample lacked uniformity. Consequently, a new media consisting of 100 ml. of one-half saturated solution of calcium sulfate containing 1.5 grams of sugar was substituted for the bacto malt and buffer solution previously used. A new sample was tested, but the results obtained were less in accord than before with more negative results. It became more apparent that lack of agreement was due to an insufficient amount of media or that the yeast culture under test was not uniform, although this sample had been ground to pass a 60-mesh sieve. Lack of time prevented further tests, but it seems highly probable that future tests in which the necessary changes will have been made will result in satisfactory results.

It is recommended that the study of yeast activity be continued.

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## REPORT ON MICROSCOPIC EXAMINATION OF FEEDS

By A. W. CRESWELL (Division of Plant Industry, Department of Agriculture, Columbus, Ohio), *Associate Referee*

The following question was asked at the Feed Control Meeting, here in Washington, two years ago: "How many States include microscopic examination in their routine analysis of feeds?" At that time the answer to this question seemed to present varied opinions, and the discussion that followed brought forth this second question: "Is it possible to obtain printed material on the procedure as developed by State Control Laboratories?"

Several years previous to this Feed Control Meeting, Ohio had experienced the difficulty of initiating the microscopic analysis of feeds. The main cause for such a difficulty was the lack of a definite source of material to begin such a procedure. Finally it was decided to use a combination of the ideas originating in this laboratory and suggestions of the Ohio Experiment Station and Ohio State University.

Therefore, the questions asked at the Feed Control Meeting in 1941

seemed most important and led the Associate Referee to seek the advice of the Secretary of the American Feed Control Association, the Referee on Feeding Stuffs of this Association, and the President of the American Feed Manufacturers Association. The results of correspondence with the representatives of these various Associations agreed with the Associate Referee that it would be a worth-while venture to seek the help and cooperation of all State Control Officials.

The first letter to each State was the preliminary step in a series of questionnaires asking for definite comments and suggestions, and led to the conclusion that 24 States and the Dominion of Canada employ full or part time personnel to examine feeds with the microscope, and that all States analyzing feeds do desire simple concise printed material on this procedure.

These opinions from control officials implied that intense interest in the microscopic examination of feeds extends throughout the States and that an exchange of suggestions on equipment and methods might lead to some similarity of opinions.

As has been stated, the questionnaire method was decided upon as the most direct and condensed form to produce results, and it was stipulated that summaries of each questionnaire would be sent to each State contributing to the survey. The first direct questions submitted to the control officials asked when microscopic analysis of feeds was inaugurated, and the name of the microanalyst in charge.

The results included in the first summary produced the following results:

Several States initiated this procedure in 1907 and 1910, and it is also interesting to note that these States were most enthusiastic concerning the procedure and were most helpful during the entire survey. The remaining States inaugurated this examination of feeds in 1913, 1920, 1930, 1938, and on up to 1942. It is assumed, therefore, that this phase of feed analysis is approximately 35 years of age. The other questionnaires throughout the year were designed—

- (1) To obtain a cross section or list of ingredients and mixed feeds as analyzed in each respective State;
- (2) To find out whether or not a collection of reference samples would be valuable to the microanalyst;
- (3) To secure a list of printed material and equipment used;
- (4) To learn what methods were found to be most practical; and finally
- (5) To see how samples are reported by microscopic examination.

In regard to the first objective on the ingredients and mixed feeds analyzed in each State, it is interesting to note the similarity of such products throughout the various States. However there are ingredients common to certain areas in the country, and they form a basis for future exchange of technic and samples.

To the second objective on the necessity of a reference collection of samples, all States contributing to the survey stated that such a collection is most valuable to the microanalyst. One State ventured to say that this reference collection seemed more authentic than illustrated pictures.

To the third objective on books, reference printed material, and equipment found to be most practical, most control officials agreed on outstanding publications that deal with microscopic technic and that are particularly related to foods and feeds, such as Experiment Station Bulletins, Information Sheets from the Food and Drug Administration and from the U. S. Department of Agriculture, Journal of the Association of Official Agricultural Chemists, and American Feed Control Definitions.

Equipment generally found to serve both beginners and trained microanalysts consists of the low-powered microscope and necessary equipment such as micro lamp, glass slides, etc.; an ordinary hand lens; a set of screens, preferably 20-40-60-mesh; additional equipment such as is commonly found in an analytical laboratory.

The fourth objective, devoted to methods used in the microscopic examination of feeds, was freely and fully discussed. Each analyst was requested to state in detail the successive steps followed when analyzing a sample. The general method is as follows:

- (a) Pour a portion of well-mixed sample on a set of 20-40-60-mesh screens.
- (b) Sift this portion of sample completely.
- (c) Identify ingredients, particularly those retained on the coarse or 20-mesh screen and compare such ingredients with those listed on tag or label.
- (d) Many ingredients pass through the 20-mesh, 40-mesh, and 60-mesh screens into the pan. Therefore, to identify all ingredients portions retained on the screens and pan should be carefully examined.
- (e) The ordinary hand lens is beneficial in examining the coarse material, and fine material should be submitted to the low-powered microscope.
- (f) The finely powdered sample necessitates the study of starch and fiber structure.
  - 60% of the States cooperating in survey included this study;
  - 30% of the States cooperating in survey state the importance of this study; and
  - 10% of the States cooperating in survey make no comment.
- (g) The microanalysis of minerals in mixed feeds bears careful investigation due to varied comments, suggestions, and even detailed drawings from Control Laboratories. Two methods of detecting mineral content in feeds seem to have preference, namely, the flotation method and the spot test.

The fifth objective on the methods and comments in reporting a sample by microscopic analysis produced answers that were as varied as those found in the annual reports of the various States. Several States use daily printed forms that combine chemical and microscopic findings in reporting a sample to the chief chemist. The matter of reporting seems to vary with each State. This completes the main objectives of the survey, yet answers to each questionnaire produced a series of interesting comments and sug-

gestions, which may be called special features, and they added a great deal of spice to the entire survey. It is impossible in this limited report to discuss the details of these special features, and they will only be classified in the order of their importance to microanalysts. The first feature included a list of ingredients known as "the hard-to-find" and methods exchanged among various States on these problem ingredients were most helpful to all. Another feature included a list of ingredients carrying the greatest foreign material and the resulting list included in the summary gave a definite cross section of the ingredients sold in the various States and the foreign material that may be found at various times during the year. A third feature dealt with the unground weed seeds found in mixed feeds. Several State control analysts report a cooperative study with feed manufacturers on this important problem.

This discussion leads to one of the most direct questions of the survey: "What do feed manufacturers and dealers think of the microscopic analysis of feeds?" The majority of States cooperating gave the impression that feed manufacturers are glad to supply samples for reference work, and for the most part feel that such analysis is most valuable in supplementing results from the chemical determination of protein, fat, and fiber.

In conclusion, the personal observations of the Associate Referee are as follows:

(1) That 24 States and Canada included this procedure in their routine analysis of feeds during the year 1942.

(2) That several States find this procedure extremely valuable after 35 years of experience.

(3) That letters from many States express a desire to initiate the microscopic examination of feeds and that printed material should be so designed as to be of value to beginners as well as trained microanalysts.

(4) That microanalysts are enthusiastic in exchanging ideas and methods, which is most encouraging in the development of new and practical methods.

#### ACKNOWLEDGMENT

The Associate Referee takes this opportunity to thank the Secretary of the American Feed Control Association, the Referee on Feeding Stuffs of this Association, and the President of the American Feed Manufacturers Association for their encouraging comments and suggestions; also, to thank State control officials including all microanalysts for making this survey possible. Special mention should be given to the Dominion of Canada for the helpful and cooperative assistance of Charlotte S. McCullough, a trained microanalyst.



REPORT ON MAGNESIUM AND MANGANESE  
IN FERTILIZERS\*

By JOHN B. SMITH, *Associate Referee*, and JOSEPH RYNASIEWICZ  
(Agricultural Experiment Station, Kingston, R. I.)

Because of increased demand for work specifically related to public needs during the war, less has been accomplished in the study of the methods assigned than in previous years. Past work has provided methods for acid-soluble magnesium and manganese that will serve, although more work is desirable to improve details.

The more difficult task of devising a practical method for measuring the "active" fraction of magnesium is incomplete. The relative usefulness of the two procedures most studied was discussed in the previous report (*This Journal*, 25, 326). It was argued that discrimination should be on the basis of the agronomic values, rather than on the basis of cost of the different ingredients.

Although the method for water-soluble magnesium includes most of that element in some processed carriers, it excludes too much of the available portion of dolomite. It was suggested that the water-soluble fraction plus one-half of the water-insoluble portion might be a simple and adequate measure. In line with this suggestion, E. T. Hord, North Carolina Department of Agriculture (private communication) analyzed 20 samples of mixed fertilizers containing from 1.25 to 6.50 per cent of acid-soluble magnesium oxide and found the water-soluble portion to be from 0.36 to 1.77 per cent. Adding one-half the insoluble to the soluble portion, he calculated that an average of 65 per cent of the acid-soluble magnesium would be classed as active.

## CITRATE-SOLUBLE MAGNESIUM

Search for a solvent similar to the neutral ammonium citrate solution used as a measure of available phosphoric acid has been in progress for some time, and for normal samples ground to pass a 20-mesh sieve it has been shown that rather satisfactory results may be obtained by substituting an acid ammonium citrate solution (*This Journal*, 23, 249), for the neutral ammonium citrate solution under the same conditions prescribed for the latter solution (*Methods of Analysis*, A.O.A.C., 1940, 24, 16). A 1-gram charge is leached with water, and the insoluble residue is extracted for 1 hour with a solution containing 6 grams of citric acid in 100 ml., adjusted to pH 4 with ammonium hydroxide. However, the solubility of dolomite is decreased somewhat by increasing the proportion of superphosphate in the mixture, probably without appreciable effect on actual

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\* Contribution No. 652 of the Rhode Island Agricultural Experiment Station.

availability; and grinding coarse particles of dolomite increases the apparent activity.

The effect of superphosphate on the solubility of dolomite has been demonstrated in previous reports (*This Journal*, 24, 268; 25, 326). Increasing the calcium from calcium nitrate had more effect than had phosphate from ammonium phosphate, but a large increment of less soluble calcium sulfate in a fertilizer mixture decreased the solubility of dolomite only to a slight extent.

Hardesty, Ross, and Adams (*This Journal*, 26, 203) have shown that the proportion of ammoniated superphosphate in the sample also affects

TABLE 1.—*Effect of varying proportions of fertilizer and dolomite on recovery of Mg by the citrate solution, and the relation of calcium and phosphate to decreased solubility of dolomite caused by fertilizer ingredients*

FER- TILIZER CHARGE <sup>1</sup>	DOLOMITE A <sup>2</sup> 0.27 GRAM				MILLRUN DOLOMITE <sup>3</sup> 65°C.				0.27 GRAM DOLOMITE A AND SINGLE SALTS					
	60°C.		65°C.		0.27 GRAM		0.17 GRAM		Ca IN 100 ML. CITRATE SOLN <sup>4</sup>	Mg RECOVERED 60°C. 65°C.		P <sub>2</sub> O <sub>5</sub> EQUIVA- LENT IN 100 ML. CITRATE SOLN <sup>5</sup>		Mg RECOV- ERED 65°C.
	Mg	Mg	Mg	Mg	Mg	Mg	Mg	Mg						
	FOUND	RECOV- ERED	FOUND	RECOV- ERED	FOUND	RECOV- ERED	FOUND	RECOV- ERED						
	gram	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	mg.	per cent	per cent	mg.	per cent
None	—	—	2.09	93	3.18	95	2 00	95	—	—	—	—	—	
0 18	2.31	74	2.50	80	2.08	62	1 71	81	23	74	79	9	80	
0 36	2 15	69	2.40	77	1 98	59	1.37	65	46	69	75	18	79	
0.55	2 09	67	2 25	72	1.78	53	1 31	62	69	66	70	27	77	
0.73	2 03	65	2 03	65	1.57	47	1 08	51	92	64	65	36	76	

<sup>1</sup> 6-8-6 fertilizer from W. H. Ross (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, ammoniated superphosphate, KCl, and quartz sand to make 1 gram, including the dolomite

<sup>2</sup> From W. H. Ross Composite from three quarries, finer than 200-mesh, 11.58% Mg.

<sup>3</sup> 12.41% Mg, 85% through 200-mesh sieve.

<sup>4</sup> No fertilizer. Ca(NO<sub>3</sub>)<sub>2</sub> equivalent to 1/4, 1/2, 3/4, and all Ca determined in the citrate solution from 0.73 gram of the 6-8-6 fertilizer and 0.27 gram of Dolomite A.

<sup>5</sup> No fertilizer. Na<sub>2</sub>PO<sub>4</sub> equivalent to 1/4, 1/2, 3/4, and all P<sub>2</sub>O<sub>5</sub> determined in the citrate solution of the full charge of 6-8-6 fertilizer and Dolomite A.

the solubility of the phosphate in the official neutral ammonium citrate solution, and that dolomite is an additional factor, decreasing the soluble phosphate in acid fertilizer mixtures containing calcium sulfate.

Further evidence is presented in Table 1. Varying weights of a simple 6-8-6 fertilizer were mixed with two different quantities of a finely ground millrun dolomite, and similar mixtures with a composite sample of 200-mesh dolomite taken from three representative quarries, designated Dolomite A. The citrate extraction was made at 60°C. as well as at the prescribed temperature, 65°C. The conditions are exaggerated as compared with normal fertilizers with respect to proportions of ingredients and the use of 200-mesh dolomite. All results were corrected by subtracting the small quantities of magnesium in the fertilizer and confining the calculations to that in the dolomite. The results show that the smallest

quantity of fertilizer decreased the recovery of magnesium and that increments of fertilizer caused further decreases. Lowering the temperature of the extraction from 65° to 60°C. also decreased the recovery of magnesium. Reducing the weight of dolomite in the charge increased the proportion of magnesium dissolved. Obviously, results by this method are not independent of the proportions of ordinary components in the fertilizer. However, if only the effects of the three-fourths and the full portions of fertilizer are considered, the differences are less extreme, and they approximate the limits of practical mixtures.

As discussed in a previous paragraph the presence of soluble calcium from superphosphate has been suspected to be the principal cause of the decreased solubility of the dolomite. To demonstrate this, the citrate extract from the water-insoluble residue of 0.73 gram of the 6-8-6 fertilizer and 0.27 gram of Dolomite A was analyzed for calcium and phosphoric acid. Calcium nitrate equivalent to  $\frac{1}{4}$ ,  $\frac{1}{2}$ ,  $\frac{3}{4}$ , and the full amount of calcium found was added to a series of 100 ml. portions of the citrate solution, and a similar series was prepared containing trisodium phosphate, also based on the analyses of the extract. Dolomite A was added to each solution in 0.27 gram portions and extracted for 1 hour at 60° and 65°C. with the calcium nitrate, and at 65°C. with the sodium phosphate. The calcium was increased somewhat by the portion dissolved from the limestone, but the phosphate concentration must have remained about the same as that shown by the fertilizer residues in the previous extractions. This does not eliminate the effects of the nitrate or sodium ions, but the results (Table 1) point quite definitely to calcium as the major factor.

Although it is unlikely that coarsely ground dolomite will be used in fertilizers except for the small quantities normally present after the usual commercial grinding, any subsequent grinding of the particles in preparation of analytical samples will increase the solubility, and the trend is toward finer grinding to provide more uniform samples. In this laboratory, one of eight inspection samples with guarantees for magnesium had 0.75 per cent of that element in the portion coarser than 1 mm., calculated on the basis of the entire mixture, but none of the remainder had more than 0.1 per cent. This small number of samples is inadequate to represent the occurrence of coarsely ground dolomite, but the possibility of its use cannot be disregarded.

In an attempt to overcome the difficulty, fertilizers were mixed to contain known weights of dolomite coarser than 1 mm. as well as finer particles; 10 gram samples of the unground mixtures were boiled with 250 ml. of water to dissolve the water-soluble fraction and disintegrate aggregate particles, and filtered. The residues were dried and passed through a 1 ml. sieve; one-tenth of the fine material, taken by weight, was extracted with the citrate solution as usual. Magnesium was determined both in the water extract and in the citrate extract. The sums of the percentages were

in substantial agreement with anticipated results, but the principal difficulty is the extra time required for the procedure.

As a detail of the method it was found that magnesium could be determined in the citrate solutions by first precipitating both calcium and magnesium by the addition of an excess of ammonium phosphate followed by ammonium hydroxide to the end point of bromothymol blue. After filtration, magnesium was determined in the precipitate by the usual method for acid-soluble magnesium. It was necessary to run a blank on the citrate solution for it contained soluble magnesium.

#### WATER-SOLUBLE MAGNESIUM

This method was developed by E. R. Tobey at the Maine Agricultural Experiment Station, primarily to distinguish between water-soluble Kie-

TABLE 2.—Commercial products used in study of water-soluble Mg

SAMPLE	Mg	MECHANICAL ANALYSIS						
		COARSER THAN 40 MESH	40-60 MESH	60-80 MESH	80-100 MESH	100-200 MESH	200-300 MESH	FINER THAN 300 MESH
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Sulfate of potash mag- nesia	11.45	—	—	—	—	—	—	—
Magnesium oxide	55.09	—	—	0.7	1.9	3.6	6.9	86.9
Calcined magnesite	45.91	—	—	—	2.2	20.4	23.1	54.3
Selectively calcined dolo- mite	17.03	19.0	18.2	8.7	8.0	17.5	11.2	17.4
Dolomite 1	11.39	1.0	3.7	8.0	9.5	32.6	14.5	30.7
Dolomite 2	11.58	17.3	33.8	12.6	10.4	16.8	4.9	4.2

serite and insoluble dolomite, the carriers in vogue when the method was first suggested. The procedure consists of boiling a 1-gram sample with 350 ml. of water for one hour, and the determination of magnesium in an aliquot. After collaborative study (*This Journal*, 21, 277; 24, 268), the method was revised, published in *This Journal*, 25, 326, and adopted as a tentative method. In practice it has recovered most of the magnesium from water-soluble sources and substantial portions of the magnesium added as oxides and as dolomite, because of the solubility of the products developed by the reactions of those compounds in the curing pile and during the extraction procedure.

The further studies of the method described in this report were designed to minimize the effects of curing and to limit the effects, so far as possible, to reactions during the extraction. To demonstrate the solubility of the reaction products mentioned most frequently, mono-, di-, and trimagnesium phosphates and magnesium ammonium phosphate were chosen.

These were taken from reagent-grade chemicals analyzed for magnesium. Commercial products representing the types of carriers in current use were secured from manufacturers, and they are listed in Table 2 with the percentages of magnesium and the mechanical analyses. All are domestic products familiar to those in the fertilizer trade. The magnesium oxide

TABLE 3.—*Effect of varying proportions of superphosphate and sulfate of ammonia in the fertilizer on recovery of magnesium from various carriers by boiling 1 gram samples with 350 ml. of water for 1 hour. All samples contained 1.21% Mg, divided equally between the carriers where two were used*

SAMPLE	FERTILIZERS*					
	3.5-4-4	3.5-8-4	3.5-12-4	3.5-6-5	6.5-6-5	9.5-6-5
	RECOVERY OF MAGNESIUM					
	per cent	per cent	per cent	per cent	per cent	per cent
Monomagnesium phosphate	91	100	102	91	90	93
Dimagnesium phosphate	97	91	107	88	99	94
Trimagnesium phosphate	81	84	80	74	74	72
Magnesium ammonium phosphate	99	109	104	97	98	98
Sulfate of potash magnesia	90	105	93	98	105	97
Magnesium oxide	77	84	86	84	95	89
Calcined magnesite	60	63	64	60	65	95
Selectively calcined dolomite	33	40	50	46	46	49
Dolomite 1	11	14	17	10	14	16
Dolomite 2	7	11	15	7	7	7
Sulfate of potash magnesia						
with magnesium oxide	77	83	88	84	95	90
with calcined magnesite	88	81	97	75	84	79
with selectively calcined dolomite	76	77	88	63	75	72
with Dolomite 1	55	58	61	52	60	55
Dolomite 1						
with magnesium oxide	36	43	45	44	49	52
with calcined magnesite	44	47	54	36	39	41
with selectively calcined dolomite	26	33	41	22	27	34

\* Composed of sulfate of ammonia, a composite sample of 8 superphosphates, 20.67%  $P_2O_5$ ; muriate of potash, 60.50%  $K_2O$ ; cocoa shell meal, the magnesium carrier, and quartz sand to make required weight.

is a finely divided product of rather high purity obtained from sea water. The calcined magnesite, which contains about 9 per cent of calcium, is made by heating the natural mineral. It is not so finely divided as the magnesium oxide, but it passes a 100-mesh sieve and over one-half is finer than 300-mesh. Selectively calcined dolomite is made by heating dolomite at a temperature to decompose the magnesium carbonate with minimum decomposition of the calcium carbonate. The product chosen to represent this type of carrier is less finely divided than the oxides, and contains 27.8 per cent of calcium. Of the two millrun dolomites, Dolomite 1 is more finely ground than Dolomite 2, but neither is extreme with respect to particle size.

Each magnesium carrier was mixed with six simple fertilizer mixtures varying in the proportions of superphosphate and of sulfate of ammonia. Other mixtures contained pairs of carriers in which each supplied one-half the magnesium. All mixtures contained 1.21 per cent of magnesium. These are listed in Table 3. The difference in potash between the superphosphate and the sulfate of ammonia series was an error that destroys a strict comparison of the two series, but previous work with the method has shown no effect of muriate of potash on water-soluble magnesium. Before the carriers of magnesium were added, the fertilizers were ground to pass a 1-mm. sieve. As the sample of magnesium oxide had become aggregated in small lumps during storage, it was necessary to rub it gently with the fertilizers in a porcelain mortar to make a uniform mixture. Grinding was avoided, but to assure fair comparison the other mixtures were treated similarly. Analyses of the mixtures with and without this treatment showed increased solubility of the magnesium oxide, but this was not the case with any other carrier. The mixtures were stored at room temperature while the analyses were in progress, but repetition at intervals showed no significant changes during approximately 18 months, and new mixtures made from the same ingredients toward the end of the period produced results similar to those from the stored samples. Small quantities of magnesium in the fertilizers without the carriers were subtracted before the recovery of added magnesium was calculated.

The recoveries are reported in Table 3 as percentages of the magnesium added. This method helps to clarify relationships, but it exaggerates the effect of analytical errors. Differences of less than 10 per cent are probably insignificant.

The results show almost complete solubility of the magnesium in the probable reaction products except for trimagnesium phosphate, from which 72–84 per cent was recovered. Varying proportions of superphosphate and sulfate of ammonia in the fertilizers had little effect on the solubility of this group of compounds. Apparently as much as 1.2 per cent of magnesium from water-insoluble compounds can be recovered from a fertilizer if it is converted to the phosphates, unless trimagnesium phosphate becomes a major component. It is quite possible that the trimagnesium phosphate in reaction mixtures may form in smaller and more soluble crystals than those used in this study, for there is no evidence that the solutions analyzed were saturated.

Among the commercial products, the sulfate of potash-magnesia was almost completely dissolved. The magnesium oxide returned from 77 to 95 per cent of the magnesium supplied; calcined magnesite gave recoveries of 60–65 per cent, except that 95 per cent was soluble in the 9.5–6–5 mixture; and from the selectively calcined dolomite from 33 to 49 per cent of the magnesium was recovered. In the three carriers the magnesium is present as the oxide, and the reactivity appears to be correlated chiefly

with particle size. The effects of increasing the phosphate and sulfate of ammonia are not entirely consistent, but the trend is toward increased recoveries. The greatest difference is a recovery of 95 per cent from calcined magnesite with 9.5-6-5 fertilizer as compared with 60-65 per cent from the other mixtures. These results were obtained from replicate mixtures, but they differ so greatly from the others that further verification is needed before acceptance.

As in previous trials, small quantities of magnesium were obtained from dolomite. More was dissolved from the more finely ground product, and there was a trend toward increased decomposition caused by increasing the sulfate of ammonia or the superphosphate. If it is assumed that the dolomite in the selectively calcined product was originally somewhat similar to Dolomite 1, there was an increase in reactivity from calcination for it supplied more water-soluble magnesium than did the untreated dolomite, although the latter had a much greater proportion of particles finer than 100-mesh. Fineness of the dolomite is not a major factor in this method.

Aside from analytical errors, recoveries from mixtures of two ingredients were not greatly different from the averages for those ingredients used alone.

As a laboratory procedure the method is simple, the results are fairly consistent, and the effects of varying the phosphate and sulfate of ammonia in the fertilizer are not very great. However, as has been stated in previous reports, the results are difficult to interpret from the agronomic point of view. The processed materials are usually considered to be quite available in acid soil, although more proof may be desirable for some conditions. Dolomites of the types represented in this study are expected to be at least 50 per cent available under average conditions. If these beliefs are correct, the method gives too low results except for water-soluble carriers and the very finely divided magnesium oxide. Larger recoveries might be expected from these ingredients added before the usual commercial curing processes, but not from the so-called dry mixes. The Associate Referee has suggested that one-half the water-insoluble magnesium be added to the water-soluble portion to provide a fairer value for "active" magnesium. This suggestion, based on the supposition that the insoluble magnesium would be chiefly in dolomite, would not hold so well for products in the insoluble fraction, such as the oxides that are believed to be more than 50 per cent available during a single growing season. However, such a calculation would be a better approximation of availability than the elimination of all water-insoluble magnesium.

As details of the method, the effects of time of extraction and volume of water were studied. Since the oxides must be converted to the phosphates or other compounds to become soluble, it seemed logical that concentration of soluble phosphates or the volume of solvent might be impor-

TABLE 4.—*Effect of volume and time on solubility of commercial carriers of MgO in boiling water (expressed as % of Mg recovered from 1-gram charge)\**

Extraction time (minutes)	6	10	30	30	30	30	60	60	60	60
Volume of water (ml.)	10	10	50	100	200	350	50	100	200	350
Carriers	Mg Recovered (%)									
Magnesium oxide	85	—	81	84	76	89	85	87	85	89
Calcined magnesite	—	40	47	47	46	—	62	51	51	52
Selectively calcined dolomite	—	33	35	36	36	—	41	37	38	35

\* All carriers mixed with the 3.5–8–4 fertilizer described in Table 3, at rates to supply 1.21% Mg.

tant factors. However, the data in Table 4 indicate very little difference for volumes of water varying from 10 to 350 ml., or for time periods of from 10 to 60 minutes.

#### ACID-SOLUBLE MAGNESIUM

Although little new work has been done with this topic, data from previous collaborative analyses justify certain recommendations in preparation for the 1945 publication of methods.

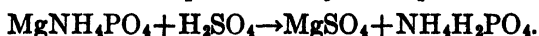
The Referee on Fertilizer has suggested elimination of methods where possible to save space. Method I for acid-soluble magnesium (*Methods of Analysis, A.O.A.C.*, 1940, 35, 52), includes precautions omitted from subsequent methods, and separates magnesium from calcium as the sulfates in an alcohol solution. It has served as a reference method in the development of shorter procedures that have given satisfactory results, and apparently is seldom used since the adoption of those methods. As an official method, approval for deletion must be voted at two meetings, and this is recommended in this report for first action. Portions of the second paragraph and the final paragraph of the method must be added to Method II as these include the correction for manganese and other directions applicable to both procedures. These are noted among the recommendations at the end of this report.

Method III (*Ibid.*, 36, 54) is a volumetric modification that has had three successful collaborative trials and was adopted as official (first action) in 1940. Although collaborative results in 1941 justified recommendation of final action, this step was not taken because of suggestions for improvement in the choice of an indicator. Comparisons of indicators have been made in this laboratory and suggestions for a better selection are made in the subsequent paragraph, but the change cannot be recommended until it has received collaborative study. Therefore, although the change is contemplated, the method is recommended for final action next year.



Methyl orange, the historic indicator for the procedure, has given very satisfactory results, but the color change is less distinct than is desired. Collaborators have recommended methyl red, and a mixture of methyl orange and xylene cyanole. The latter was incorrectly stated to be methyl red and xylene cyanole in the previous report. Marsden<sup>1</sup> has suggested a mixture of bromocresol green with neutral red for the same purpose.

The reaction involved is represented by the equation:



The pH of the theoretical end point was determined in three ways. A clear solution of equimolecular mixtures of the reactants at concentrations

TABLE 5.—Comparison of indicators for volumetric method for acid-soluble Mg

INDICATOR	MG. IN FERTILIZER <sup>5</sup>			TITRATION <sup>6</sup> FROM INDICATOR END POINT TO pH 4.40	COLOR AT pH 4.40
	NO. 1	NO. 3	NO. 4		
	per cent	per cent	per cent	ml. 0.1 N	
Methyl orange <sup>1</sup>	1.80	2.27	2.28	Acid 0.06	yellow
Methyl orange, xylene cyanole <sup>2</sup>	1.82	2.24	2.26	Acid 0.13	green
Neutral red, bromocresol green <sup>3</sup>	1.72	2.24	2.24	Acid 0.05	blue
Methyl red <sup>4</sup>	1.63	2.12	2.12	Base 0.36	orange

<sup>1</sup> 0.1 gram methyl orange, 100 ml. water. Red to yellow.

<sup>2</sup> 0.2 gram methyl orange, 0.28 gram xylene cyanole, 100 ml. 50% alcohol. Purple to gray to green.

<sup>3</sup> 0.02 gram neutral red, 0.2 gram bromocresol green, 100 ml. 50% alcohol. Orange to colorless to blue.

<sup>4</sup> 0.2 gram methyl red, 100 ml. 60% alcohol. Red to yellow.

<sup>5</sup> Collaborative samples, and averages of collaborative results with methyl orange discussed in *This Journal*, 25, 326. Mg calculated from ingredients: 1.74, 2.24, 2.28%.

<sup>6</sup> 0.1 gram  $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$  with asbestos similar to that from pad in Gooch crucible.

representing an aliquot from a fertilizer containing 3 per cent magnesium has a pH of 4.38 by a glass electrode. Similarly, a solution of equivalent weights of the reaction products had the pH value, 4.34. Magnesium ammonium phosphate dissolved in a calculated small excess of standardized sulfuric acid and titrated with sodium hydroxide equivalent to the excess acid, had a pH of 4.43. The chemicals used were reagent grade, without further purification other than drying at 100°C., but the results agree sufficiently well for the purpose.

Three fertilizers analyzed by the collaborators in 1941 were reanalyzed in this laboratory, the indicators suggested being used. Also, aliquots of a solution of reagent-grade magnesium ammonium phosphate dissolved in sulfuric acid were titrated with sodium hydroxide to the most obvious end points of the indicators. The solutions were then further titrated with 0.1 N acid or base to pH 4.40, a glass electrode being used. The results, listed in Table 5, are best with methyl orange or the neutral red-bromo-

<sup>1</sup> *J. Soc. Chem. Ind.*, 60, 20 (1941).

cresol green mixture. Methyl orange with xylene cyanole appears to be satisfactory and has a sharper end point than does methyl orange alone. Methyl red was the least satisfactory. Neutral red-bromocresol green gave the sharpest and most discernible change, and it is the indicator preferred by the junior author.

#### MANGANESE

No work on manganese was done during the year, but certain recommendations based on previous work are desirable before the publication of the 1945 revision of *Methods of Analysis*, A.O.A.C.

The volumetric periodate method, now official (first action) has proved less popular with collaborators than the equally accurate bismuthate method. Two volumetric methods are unnecessary, and deletion of the periodate method is recommended. The bismuthate volumetric method, also official (first action) has been studied collaboratively twice (*This Journal*, 24, 268; 25, 326) and should receive final action.

The colorimetric periodate method now official (first action) has been studied collaboratively at four separate times, with satisfactory results. There are questions as to its application to samples with more than 5 per cent manganese to which it is now restricted, but the present limit is conservative. This method, also, should receive final action.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the official method for acid-soluble magnesium (*Methods of Analysis*, A.O.A.C., 1940, 35, 52) be deleted with the exception of the portion beginning in the second paragraph with the words "Transfer the precipitate" and continuing through the third paragraph. That the sentence in the third paragraph, stating "If alcoholic filtrate is clear, the  $\text{Ca}_3(\text{PO}_4)_2$  will not exceed 0.3 mg. and may be neglected," be deleted. That the entire portion retained be transferred to Method II, 54, to replace the directions in that method, beginning with "Transfer the precipitate to a small filter" (first action).

(2) That Method III, Volumetric Modification of the Method for Acid-soluble Magnesium (*Methods of Analysis*, A.O.A.C., 1940, 36, 54), adopted as official (first action), (*This Journal*, 24, 47) and studied collaboratively on three occasions, be adopted as official (final action).

(3) That methods for acid-soluble magnesium and magnesium activity be further studied.

(4) That the volumetric periodate method (*Methods of Analysis*, A.O.A.C., 1940, 37, 56, 57) be deleted.

(5) That the bismuthate volumetric method (*This Journal*, 24, 268), adopted as official (first action), (*This Journal*, 25, 49), and studied col-

\* For report of Subcommittee A and action by the Association, see *This Journal*, 27, 45 (1944).

laboratively on two occasions, be labeled "For manganese salts and fertilizers," and be adopted as official (final action).

(6) That the colorimetric method for the determination of manganese (*Methods of Analysis*, A.O.A.C., 1940, 37, 58), adopted as official (first action), (*This Journal*, 24, 47), and corrected editorially (*This Journal*, 25, 49, 79) be adopted as official (final action).

(7) That methods for acid-soluble manganese be further studied.

## REPORT ON ACID- AND BASE-FORMING QUALITY OF FERTILIZERS\*

By H. R. ALLEN, *Associate Referee*, and LELAH GAULT (Kentucky Agricultural Experiment Station, Lexington, Ky.)

Collaborative work conducted this year was in part a continuation of last year's study (*This Journal*, 26, 68). The use of 0.5 *M* sodium carbonate, titration by means of the glass electrode, and determination of basicity of coarser-than-20-mesh material in mixed fertilizers were studied. In addition, the determination of basicity of a mixed fertilizer with a high dolomite content was investigated and a comparison was made of ashing temperatures of 500° and 600°C. to determine the relative amounts of nitrogen in a mixed fertilizer volatilized at these temperatures.

### DIRECTIONS FOR COLLABORATIVE STUDY

I. Mix each of Samples 1 and 2 thoroughly on glazed paper or oilcloth. Determine acid- or base-forming quality for Samples 1 and 2 by the tentative procedure (*Methods of Analysis*, A.O.A.C., 1940, 59, p. 37), using a 1 gram portion. Duplicate this procedure for Sample 2, using a 0.5 gram portion. Make another determination for Sample 2, using a 1 gram portion and 32 ml. of normal HCl in place of 30 ml. Make a corresponding blank titration. In all analyses of Sample 2, after digestion in normal HCl and filtering, test residue for undecomposed carbonate as follows: Spread filter paper or asbestos pad on watch-glass and add a few drops of (1+1) HCl to the residue. Note whether effervescence takes place. Omit corrections for acidity of nitrogen and of insoluble  $P_2O_5$ .

II. Dissolve 53 grams of anhydrous  $Na_2CO_3$  or 143 grams of  $Na_2CO_3 \cdot 10H_2O$ , and 25 grams of sucrose, in water and dilute to 1 liter. Duplicate Procedure I, using 10 ml. of this 0.5 *M*  $Na_2CO_3$  solution and 20 ml. of normal HCl. Use 22 ml. of normal HCl in place of 32 ml. New blank titrations are required for the 20 and 22 ml. of normal HCl. Calculation is unchanged.

III. Apply the tentative method to Samples 1 and 2, using a 1 gram portion, but omit the filtering step before titration. Cool solution and titrate to pH 4.3, using a glass electrode apparatus and a continuous stirrer. (The titration may be made in the 150 ml. beaker.) Use 30 ml. of normal HCl for Sample 1 and 32 ml. for Sample 2, with corresponding blank titrations. A glass electrode apparatus with a lockdown key is desirable for satisfactory work.

\* This investigation, made in connection with a project of the Kentucky Agricultural Experiment Station, is published by permission of the Director.

IV. Apply the tentative method to Sample 1 in duplicate, using a 1 gram portion, through evaporation on the sand bath. Make another set of duplicate determinations, using 0.5 *M*  $\text{Na}_2\text{CO}_3$  solution. Ignite one molar and one 0.5 *M*  $\text{Na}_2\text{CO}_3$  determination in furnace at 250° C. and raise temperature to 500° C., holding this temperature for 1 hour. Repeat this procedure with the other molar and 0.5 *M*  $\text{Na}_2\text{CO}_3$  determination, but raise the final temperature to 600° C. Loosen ashed samples from beakers with a spatula and transfer contents to corresponding Kjeldahl flasks with spatula and brush. Determine nitrogen by one of the official methods, modified to include nitrate nitrogen. A blank determination should be made.

V. For Samples 3 and 4. Mix each of the unground samples thoroughly on glazed paper or oilcloth, breaking up any caked lumps, but do not reduce particle size of components. After mixing each sample, spread out sample uniformly and weigh accurately three 100-gram portions. To get uniform distribution, take material for each 100-gram portion from 12 to 15 places as follows: Use a piece of light sheet aluminum or cardboard about 3×3 inches and turn up 2 opposite sides to make a flat boat with open ends. Slide boat under sample, chose one end by holding a piece of sheet aluminum or cardboard firmly against it and transfer contents to weighing container. After weighing one 100-gram portion, re-mix sample, spread out, and repeat the operation for a second and third 100-gram portion. Number the samples 3a, 3b, 3c, and 4a, 4b, 4c, respectively.

Transfer each sample (100 grams) to a 5-inch, 20-mesh sieve and separate by dry sieving. Place the portion coarser than 20-mesh in a 400 ml. beaker, add 100 ml. of water, and let stand 10 minutes, stirring at least 4 times with a glass rod; transfer the sample to the 5-inch, 20-mesh sieve, which is supported by the walls of a 2 liter Pyrex beaker; wash with a stream of distilled water from an overhead source to a volume of 1500 ml. as determined by a mark on the side of the beaker. Take care to direct the water stream uniformly on all parts of the sample. Place the sieve containing the washed, coarser-than-20-mesh portion on a watch-glass and dry in an oven at 70°–75° C. for 5 hours. Remove from oven and let stand in contact with laboratory atmosphere for at least an hour. Remove all the sample from the sieve, with the aid of a spatula and weigh. Prepare sample for analysis by grinding it to pass a 0.5 mm.- or 35-mesh sieve and mix well. Analyze by the tentative method, using a 1 gram portion. Multiply the results in pounds  $\text{CaCO}_3$  per ton by weight of the coarser-than-20-mesh portion divided by 100 (the amount of coarser-than-20-mesh material to be subtracted from the ash basicity of the whole sample before corrections are applied for nitrogen and insoluble  $\text{P}_2\text{O}_5$ ). Determine moisture on Samples 3a, 3b, 3c, and 4a, 4b, and 4c, drying at 100° C. for 5 hours.

Report all basicity results in pounds  $\text{CaCO}_3$  per ton.

The following analysts collaborated in this work.

#### COLLABORATORS

- (1) W. R. Austin, Armour Fertilizer Works, Nashville, Tenn.
- (2) C. R. Byers, Armour Fertilizer Works, Carteret, N. J.
- (3) R. D. Caldwell, Armour Fertilizer Works, Atlanta, Ga.
- (4) Mary C. Fox, Davison Chemical Corporation, Baltimore, Md.
- (5) T. L. Ogier, Agricultural Experiment Station, College Station, Texas.
- (6) B. L. Samuel, and R. C. Berry, Department of Agriculture, Richmond, Va.
- (7) A. F. Spelman, Agricultural Experimental Station, Amherst, Mass.
- (8) Ruth D. Tosh, American Agricultural Chemical Co., Carteret, N. J.
- (9) H. R. Allen and Lelah Gault.

## COLLABORATIVE SAMPLES

Sample 1 was a mixture containing 90 per cent of a mixed fertilizer base and 10 per cent of a dolomite; Sample 2 contained 60 per cent of a mixed fertilizer base and 40 per cent of dolomite. The amount of dolomite in Sample 2 would not usually be found in a mixed fertilizer, but this quantity may be present in washed, coarser-than-20-mesh portion if an excessive amount of coarser-than-20-mesh dolomite was incorporated in the mixed fertilizer. It has been found in this laboratory that in determining the basicity of such samples and using a 1-gram portion and 30 ml. of normal hydrochloric acid there is sufficient excess acid present to react with all the carbonate in the sample, but the reaction does not always proceed to completion because of the low acid concentration. A back titration is obtained with 0.5 *N* sodium hydroxide which results in a low basicity figure. The presence of undecomposed carbonate is shown by effervescence when the residue, after filtration, is treated with hydrochloric acid. In such cases, or in determination of basicity of a dolomite, it is necessary to increase the amount of acid or to reduce the size of the sample. Collaborative work on Sample 2 compares these two procedures.

Separate portions of 400 grams each of unground Samples 3 and 4 were prepared for each collaborator, to insure against non-uniformity of sample. Sample 3 contained 390 grams of a mixed fertilizer base and 10 grams of dolomite, all of which was between 10 and 20 mesh in particle size. Sample 4 contained 380 grams of the same base and 20 grams of the same dolomite. The basicity of the washed, coarser-than-20-mesh portion of the base used in Samples 3 and 4 analyzed 14 pounds of calcium carbonate equivalent and the basicity of the amount of dolomite used was 51 pounds of calcium carbonate equivalent for Sample 3 and 102 pounds for Sample 4.

Tables 1 and 2 contain collaborators' results on Samples 1 and 2, and Tables 3 and 4 contain collaborators' results on Samples 3 and 4.

TABLE 1.—*Collaborators' results on Sample 1 (ash basicity in pounds CaCO<sub>3</sub>/ton)*

COLLABORATOR	TENTATIVE METHOD	0.5 <i>M</i> Na <sub>2</sub> CO <sub>3</sub>	GLASS ELECTRODE TITRATION
1	348*	341*	351*
2	300	298	—
3	307	—	—
4	323	296	—
5	310	285	304
6	302	295	302
7	310	311	—
8	351*	325*	—
9	320	305	312
Average	310	298	306

\* Omitted from average.

TABLE 2.—*Collaborators' results on Sample 2 (ash basicity in pounds CaCO<sub>3</sub>/ton)*

COLLABORATOR	M Na <sub>2</sub> CO <sub>3</sub>			0.5 M Na <sub>2</sub> CO <sub>3</sub>		
	1 GRAM AND 30 ML. N HCl	1 GRAM AND 32 ML. N HCl	0.5 GRAM AND 30 ML. N HCl	1 GRAM AND 20 ML. N HCl	1 GRAM AND 22 ML. N HCl	0.5 GRAM AND 20 ML. N HCl
1	761	761	790	—	768	—
2	698	743	760	715	725	740
3	—	750	—	—	750	—
4	662	763	763	631	649	777
5	728	721	739	659	686	675
6	730	737	775	725	758	785
7	705	720	705	—	713	713
8	703	757	793	704	730	792
9	723	758	775	685	750	780
Average	714	746	762	687	726	752

TABLE 3.—*Collaborators' results on ash basicity of material coarser than 20-mesh (pounds CaCO<sub>3</sub>/ton)*

COLLABORATOR	SAMPLE 3		SAMPLE 4	
	AVERAGE*	MAXIMUM VARIATION†	AVERAGE*	MAXIMUM VARIATION†
1	65	4	101	4
2	54	8	105	6
3	52	1	90	16
4	60	8	106	14
5	62	10	109	1
6	67	3	108	10
7	64	14	106	0
8	55	7	98	9
9	64	1	110	7

\* Average of 3 determinations.

† In 3 determinations.

TABLE 4.—*Collaborators' results in grams on weights of washed, coarser-than-20-mesh portions*

COLLABORATOR	SAMPLE 3		SAMPLE 4	
	AVERAGE*	MAXIMUM VARIATION†	AVERAGE*	MAXIMUM VARIATION†
1	14.15	0.61	16.80	0.83
2	12.72	0.50	16.75	1.15
3	13.38	0.33	14.89	3.10
4	13.69	1.96	17.53	1.76
5	14.24	4.15	17.30	0.56
6	14.14	0.68	19.12	4.19
7	13.87	3.03	16.14	1.06
8	12.02	1.85	14.02	2.10
9	15.07	2.91	16.54	2.39

\* Average of 3 determinations.

† In 3 weights.

## DISCUSSION OF RESULTS

*Sample 1.*—Seven of the collaborators obtained very uniform results by the tentative method on Sample 1, and those analysts who used the glass electrode titration obtained results in good agreement with their figures for the tentative method. The use of 0.5 *M* sodium carbonate gave somewhat lower results.

Six collaborators found nitrogen in Sample 1 ashed at 500° C. with molar sodium carbonate varying from .07 to 0.24 per cent, with an average of 0.16 per cent. The average of the nitrogen found by the same collaborators on Sample 1 ashed at 600° C. was .04 per cent. Two collaborators reported no nitrogen in Sample 1 ashed at 500° or 600° C. The results were similar with use of the 0.5 *M* sodium carbonate. It is evident that 500° is not high enough to volatilize all the nitrogen in the sample.

*Sample 2.*—As expected, the basicity results on Sample 2, using a 1-gram portion and 30 ml. of normal hydrochloric acid, were low and erratic. Most of the collaborators reported effervescence when the residue was treated with hydrochloric acid. Much more uniform results were obtained with the 32 ml. of normal hydrochloric acid and with the 0.5 gram sample. The use of the 0.5 gram sample for larger amounts of dolomite seems preferable to the use of increased amount of acid. The results with 0.5 *M* sodium carbonate were much less uniform than with the molar sodium carbonate and it seems that study of the former should be discontinued. The decreased amount of sucrose in the 0.5 *M* sodium carbonate results in less carbon in the ashed samples. This is a distinct advantage and the use of a molar sodium carbonate solution containing 25 grams of sucrose per liter might be desirable.

*Samples 3 and 4.*—A major factor in determination of basicity of material coarser than 20-mesh in mixed fertilizers is the difficulty of obtaining uniform portions from the unground samples. To check on this, the collaborators were sent 400 gram portions of Samples 3 and 4 and asked to weigh three 100-gram portions of each to determine the basicity of the coarser-than-20-mesh portion by the proposed method. The collaborators obtained duplicate results for 3 determinations on each sample that agreed within 10 pounds of calcium carbonate equivalent except in 3 of 18 cases for the 2 samples; 6 of the 9 collaborators agreed within 7 pounds of calcium carbonate equivalent on Sample 3, and 7 of them agreed within 9 pounds on Sample 4. The results are in good agreement with computed values obtained from separate determination of basicity of the washed, coarser-than-20-mesh portion of the base and of the dolomite used.

The weights of the washed, coarser-than-20-mesh portions of the same sample varied considerably for some collaborators as did the weights reported by different collaborators. The superphosphate was the most variable component of the samples in particle size and the variation in

weights was probably caused by difficulty of sampling this component uniformly.

#### SUMMARY

Satisfactory agreement was obtained by collaborators with the tentative method and with titration by use of the glass electrode. A 0.5 gram sample gave more uniform results than the increased amount of acid in determination of basicity of a mixed fertilizer containing an excessive quantity of dolomite. More uniform results were obtained with molar than with 0.5 *M* sodium carbonate. Most collaborators found an appreciable amount of nitrogen in the sample ashed at 500° C. Good agreement was obtained by collaborators in determination of basicity of coarser-than-20-mesh material in mixed fertilizers.

#### RECOMMENDATIONS\*

It is recommended—

- (1) That the following optional method be adopted as tentative.

##### *Optional Method*

Proceed as in 60, p. 38, *Methods of Analysis*, A.O.A.C., 1940, through the addition of 50 ml. of water and 30 ml. of normal HCl and digestion on hot plate or steam bath for 1 hour. Cool to room temperature and without filtering titrate the solution in the 150 ml. beaker with 0.5 *N* NaOH to pH 4.3, using a glass electrode apparatus or other standard means of electrometric titration, and a continuous stirrer. Make the usual blank titration, using the glass electrode. Calculate results as directed in 60.

- (2) That in 60, p. 38, line 11, the figures 500–600° be changed to 575–600°.

- (3) That the following method for elimination of basicity due to material coarser than 20-mesh in mixed fertilizers be adopted as tentative.

Mix the unground sample, breaking up any caked lumps. Spread out sample uniformly and weigh a 100-gram portion, taking the material from about 12 places as follows: Use a piece of light sheet aluminum or cardboard about 3×3 inches and turn up two opposite ends to make a flat boat with open ends. Slide boat under sample, close one end by holding a piece of aluminum or cardboard against it, and transfer contents to weighing container. Transfer the sample to a 5 inch, 20-mesh sieve and separate by dry sieving. Place the portion coarser than 20-mesh in a 400 ml. beaker, add 100 ml. of water and let stand for 10 minutes, stirring 4 times with a glass rod; transfer the sample to the 5 inch, 20-mesh sieve which is supported by the walls of a 2 liter Pyrex beaker, and wash with a stream of distilled water from an overhead source, preferably with some pressure, to a volume of 1500 ml., as determined by a mark on the side of the beaker. Place the sieve containing the washed, coarser-than-20-mesh portion on a watch glass and dry in an oven at 70–75° for 5 hours. Remove from oven and let stand in contact with laboratory atmosphere for at least 1 hour. Remove all the sample from the sieve, grind sample to pass a 0.5 mm. or 35-mesh sieve and mix well. Analyze by the

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 27.



tentative method, 59-60, using a 1-gram portion. After filtering, test residue with (1+1) HCl, and if the presence of undecomposed carbonate is indicated by effervescence, repeat the determination, using a 0.5 gram sample. Multiply the results in pounds  $\text{CaCO}_3$ /ton by weight of the coarser-than-20-mesh portion divided by 100. (This is the amount of coarser-than-20-mesh material to be subtracted from the ash basicity of the whole sample before corrections are applied for acidity due to nitrogen and insoluble  $\text{P}_2\text{O}_5$ .)

(4) That in line 6 of par. 60, p. 38, after the words "except for unmixed nitrate salts," the words "or except for mixed fertilizers containing considerable nitrate nitrogen" be added (final action).

(5) That the use of a molar sodium carbonate solution containing 25 grams of sucrose per liter be studied.

(6) That the determination of basicity of dolomite be studied, particularly in relation to the size of the sample.

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## REPORT ON CALCIUM AND SULFUR

By GORDON HART (Department of Agriculture, Tallahassee, Florida),  
*Associate Referee*

No work was done by the Referee in 1942 and 1943.

It is recommended that the gravimetric method on which collaborative work was done in 1939, 1940, and 1941, be made tentative. This method (4) was published in *This Journal*, 24, 300.

There is now no tentative gravimetric method.

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## REPORT ON PLANTS

By E. J. MILLER (Agricultural Experiment Station, East Lansing, Mich.), *Referee*

Owing to war conditions the only reports that have been received from associate referees are the following:

- (1) Report on Iron in Plants, by E. J. Benne and A. Joyce Snyder.
- (2) Report on Chlorophyl in Plant Tissue, by E. J. Benne, Dorothy I. Rose, and C. L. Comar.

## RECOMMENDATIONS\*

Under the present circumstances it is possible to make only the following recommendations:

- (1) That the studies on sampling be continued.
- (2) That the studies on iodine and boron be continued.
- (3) That the studies on carbohydrates be continued.

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 27, 49 (1944).

(4) That the studies on copper and cobalt be continued and collaborative work be initiated as soon as conditions permit.

(5) That the work on carotene and chlorophyll be continued.

(6) That the work on iron be continued.

(7) That the thiocyanate method for the colorimetric evaluation of iron, *Methods of Analysis, A.O.A.C.*, 1940, 126, 7, be dropped.

(8) That the study of the o-phenanthroline procedure for the colorimetric and the titanous chloride method for the titrametric evaluation of iron be continued with the view to adopting them as official methods as soon as necessary conditions have been met.

(9) That further study be given to the ashing of plant material and extraction of the ash for the analysis of iron.

(10) That the calibration of a photoelectric colorimeter by means of a plant extract as described in the following report by Benne, Rose, and Comar be given further study and applied to different makes of instruments.

(11) That the two methods detailed in the "Report on Chlorophyll in Plant Tissue" by Benne, Rose, and Comar be adopted as tentative methods: (a) Photoelectric colorimetric method for total chlorophyll only; (b) spectrophotometric method for total chlorophyll and the *a* and *b* components.

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## REPORT ON CHLOROPHYL IN PLANT TISSUE\*

By ERWIN J. BENNE, *Associate Referee*, DOROTHY I. ROSE, and C. L. Comar† (Agricultural Experiment Station, East Lansing, Mich.)

In the reports on Chlorophyll and Carotene in Plant Tissue for 1940 and 1941<sup>1</sup> it was recommended that increased study be given to the evaluation of chlorophyll. This recommendation has been followed, and although work on methods for determining carotene has been continued, only that pertaining to chlorophyll will be treated in this report.

As described in the former reports, the Petering-Wolman-Hibbard method<sup>2</sup> for evaluating total chlorophyll with a photoelectric colorimeter depends upon a commercial chlorophyll preparation as the calibration standard for the instrument. Such preparations are not only difficult to obtain at present, but as emphasized by the work of Mackinney,<sup>3</sup> Zscheile and Comar,<sup>4</sup> and others, their spectral absorption characteristics are likely to have changed radically because of the tendency of chlorophyll to un-

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† Present address: University of Florida, Gainesville.

<sup>1</sup> *This Journal*, 24, 526 (1941); 25, 573 (1942).

<sup>2</sup> *Ind. Eng. Chem., Anal. Ed.*, 12, 148 (1940).

<sup>3</sup> *J. Biol. Chem.*, 132, 91 (1940).

<sup>4</sup> *Bot. Gas.*, 102, 463 (1941).

dergo degradation during the isolation and drying processes. As is true of any absolute colorimetric method, it is necessary that the spectral characteristics of the chlorophyll calibration standard be identical with those of the substance being evaluated in the unknown solution if accurate analytical values for this pigment are to be obtained. Hence, the lack of a reliable calibration standard would be a serious drawback to this otherwise convenient method.

TABLE 1.—*Chlorophyll values by spectrophotometric method compared with those by photoelectric colorimetric method when different calibration standards were used with the latter*

LEAF TISSUE ANALYZED	SPECTRO- PHOTOMETRIC METHOD	PHOTOELECTRIC COLORIMETER CALIBRATED WITH:			
		CHLOROPHYL IN PLANT EXTRACT	CHLOROPHYL BY Z.-C. METHOD	COMMERCIAL CHLOROPHYL SAMPLE 1	COMMERCIAL CHLOROPHYL SAMPLE 2
	per cent chlorophyll a	mg. of total chlorophyll/gram of fresh leaf			
Alfalfa	72.7	2.70	3.30	3.68	4.76
American elm	72.5	1.49	1.45	2.76	4.08
Blue grass	67.1	2.80	2.78	3.38	4.88
Carrot	73.9	1.72	1.64	1.98	2.26
Clover	67.9	3.34	3.35	4.05	4.48
Corn	76.4	1.23	1.22	1.50	1.72
Grape	69.1	2.53	2.44	2.92	3.30
Lettuce	74.0	.55	.54	.67	.75
Pea	75.1	1.86	1.77	2.17	2.47
Peony	72.1	2.81	2.60	3.17	3.57
Popple	71.2	2.32	2.20	2.65	2.98
Potato	75.3	1.57	1.53	1.85	2.11
Rhubarb	73.1	1.26	1.22	1.50	1.73
Wheat	71.5	1.38	1.41	1.74	1.98

Recently Comar, Benne, and Buteyn<sup>5</sup> have shown that wide variations existed between values for total chlorophyll obtained by the spectrophotometric method of Comar and Zscheile,<sup>6</sup> and Comar,<sup>7</sup> and those obtained with the photoelectric colorimeter when different chlorophyll preparations were used as calibration standards for the latter instrument. These authors described a means of calibrating a photoelectric colorimeter for chlorophyll by use of a simple plant extract, a procedure that eliminates the necessity of isolating the chlorophyll and thereby avoids the possibility of pigment degradation.

This work appeared to be of sufficient significance to warrant further investigation; hence, it was continued and extended as reported here.

<sup>5</sup> *Ind. Eng. Chem., Anal. Ed.*, 15, 524 (1943).

<sup>6</sup> *Plant Physiol.*, 17, 198 (1942).

<sup>7</sup> *Ind. Eng. Chem., Anal. Ed.*, 14, 877 (1942).

In Table 1 the additional analytical results show a comparison of chlorophyll values obtained by the spectrophotometric method and the photoelectric colorimetric method when different calibration standards were used with the latter.

The spectrophotometer employed in this work was the Cenco-Sheard spectrophotometer described by Comar.<sup>7</sup> The instrumental details of the photoelectric colorimetric evaluations were in accordance with the Petering-Wolman-Hibbard method.<sup>2</sup> A Cenco-Sheard-Sanford photometer was used with a combination of Corning H. R. light filters Nos. 243 and 396 of standard thickness, as suggested by these authors. Full details of this method as used will be given later in the report. It will be noted that four different standards were used for calibrating this instrument: viz., three different chlorophyll preparations and chlorophyll that was not removed from solution after being extracted from the plant tissue.

The standard designated as commercial chlorophyll, Sample 1, was a research grade of chlorophyll purchased from a domestic company. According to information furnished with the material it was prepared in June 1942, and was stored in a cool place until sold. During the interim between its receipt and use by the authors (February 1943) it was stored in the dark at approximately 5°C. That designated "commercial chlorophyll," Sample 2, was a research grade of chlorophyll purchased several years ago from the same company. It had been stored for the most part under the same conditions as described for Sample 1. The chlorophyll termed "chlorophyll by Z.-C. method" was prepared in this laboratory by the method of Zscheile and Comar<sup>4</sup> in August 1941. This material had been dried at the time of preparation and stored under the conditions described for the foregoing standards until used in this work approximately 15 months later. As shown by Comar *et al.*<sup>6</sup> the absorption spectra of these preparations in diethyl ether and the calibration curves obtained by their use with a photoelectric colorimeter varied widely and accounted directly for the variable analytical values obtained.

Calibration of the instrument by means of a plant extract was accomplished as follows: A sample of fresh leaf material was extracted with 85 per cent acetone solution and filtered, the residue was washed free of green color, and the solution was made to volume as prescribed by the analytical procedure. A series of dilutions was made from this extract, and the percentage of light transmitted by the original and by each of the diluted solutions was measured with the photometer in the same manner as when a chlorophyll preparation is used as the calibration standard. An aliquot of the original acetone solution was transferred to diethyl ether, and total chlorophyll was evaluated by the spectrophotometric method of Comar and Zscheile,<sup>6</sup> and Comar,<sup>7</sup> details of which follow later in this report. From the value thus obtained the chlorophyll content of the ace-

tone extract and that of each of the diluted solutions was calculated, and the calibration curve relating concentration of chlorophyll and percentage of light transmitted, or the  $\log_{10}(100^*/T^*)$  values, was constructed as usual.

An example will serve to illustrate this calibration procedure. A 4.00-gram sample of fresh grape leaves was extracted with 85 per cent acetone solution, and the volume of the filtered extract was brought to 250 ml. According to the spectrophotometric analysis of an aliquot of this extract, it contained 4.44 mg. of chlorophyll per 100 ml. The dilutions made, the chlorophyll contents of the resulting solutions, and the percentage of light transmitted by each (photometer readings) are given in Table 2.

TABLE 2.—*Calibration of photoelectric colorimeter with chlorophyll in an acetone extract of fresh grape leaves*

DILUTION		CHLOROPHYLL CONTENT MG./100 ML.	PHOTOMETER READING (% TRANSMISSION)	$\log_{10} \frac{100}{T}$ VALUE
ACETONE EXTRACT	85% ACETONE			
ml.	ml.			
1	9	.444	80.5	.094
2	8	.888	65.9	.181
3	7	1.33	55.1	.259
4	6	1.78	47.1	.327
5	5	2.22	40.3	.395
6	4	2.66	35.1	.455
7	3	3.11	30.9	.510
8	2	3.55	27.1	.567
9	1	4.00	24.1	.618
10	0	4.44	21.2	.674

A calibration curve for chlorophyll obtained as described above by use of an acetone extract of leaf tissue from a typical plant should be valid for evaluating chlorophyll in acetone extracts of most plant tissues. The authors used such extracts of fresh leaf tissue from American elm, popple, and grape as calibration standards, in addition to those from Norway maple and tomato as previously reported. The calibration curves obtained by use of the extracts of these different tissues coincided as closely as it was possible to duplicate curves with an extract from any one tissue. This indicates that an extract of a typical leaf tissue would serve as a calibration standard for the analysis of chlorophyll in most plants. In the case of a tissue with a highly abnormal ratio between chlorophylls *a* and *b*, however, the instrument should be calibrated with an extract of this or of another tissue with a similar chlorophyll *a* to *b* ratio.

\* Per cent light transmission of solvent and solution, respectively.

Although a specific type of photoelectric colorimeter was employed in this work, similar results should be obtainable with other makes of instruments of equivalent precision if they are provided with proper light filters. Even though a spectrophotometer is unavailable this means of calibrating a photoelectric colorimeter for chlorophyll can still be used, provided arrangements can be made with another laboratory to make the measurements at 6600 and 6425Å. required to evaluate chlorophyll concentration in diethyl ether solution. The person desiring this information should prepare the ether solutions of appropriate concentrations for measurements at 6600 and 6425Å. and ship them by air express in tightly stoppered containers packed in dry ice to the laboratory making the spectrophotometric measurements. Zscheile, Comar, and Mackinney<sup>8</sup> exchanged samples satisfactorily in this way between Indiana and California, and it was found that such ether solutions can be stored for at least 3 months without change in chlorophyll values.<sup>6</sup> The original acetone extract should be diluted and the colorimetric readings made as soon as practicable after completion of the extraction in order to avoid possible loss of chlorophyll by decomposition. The chlorophyll concentrations can then be calculated and the calibration curve constructed after the spectrophotometric data are received.

It will be noted from Table 1 that the spectrophotometric and colorimetric values agreed closely when the plant extract was used as the calibration standard, but that they varied widely when the different chlorophyll preparations were used as standards. With the latter standards all the colorimetric values were higher than the spectrophotometric values, and they varied in accordance with the differences in the spectral characteristics of the standards. This increase amounted to approximately 20 per cent in all analyses with the Zscheile-Comar chlorophyll, 35 per cent with Sample 1, and more than 60 per cent with Sample 2 of the commercial chlorophyll. This is precisely what would be expected from consideration of the absorption spectra of the standard preparations, and it corroborates the earlier findings in all respects. Hence, the authors believe that calibration of a photoelectric colorimeter with a plant extract as described above, combined with careful analytical technic, will enable investigators to obtain analytical values for total chlorophyll by the colorimetric method in good agreement with those obtained by the spectrophotometric method, and that it will increase the reliability of interlaboratory comparisons. The analyses reported in this paper were made by Benne and Rose, whereas those reported in the previous paper<sup>5</sup> were made by Comar and Buteyn. The close agreement in the results obtained indicates that the methods used are satisfactory in the hands of different

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<sup>8</sup> *Plant Physiol.*, 17, 666 (1942):

workers. Total chlorophyll can be evaluated somewhat more rapidly by the colorimetric method, but if the amounts of chlorophylls *a* and *b* are desired the spectrophotometric method must be used.

It is the opinion of the Associate Referee that the methods given in detail below are the best available for analyzing plant tissue for total chlorophyll and for total chlorophyll and the percentages of the *a* and *b* components, respectively. He therefore recommends:\* (1) That they be adopted by the Association as tentative methods for this purpose; and (2) that the calibration of a photoelectric colorimeter by means of a plant extract as described above be given additional study and applied to different makes of instruments.

### CHLOROPHYLL

#### *Photoelectric Colorimetric Method for Total Chlorophyll Only<sup>2,5</sup>*

#### 1

##### APPARATUS

(a) *Filter paper*.—A good grade of quantitative paper to fit the Büchner funnels if used.

(b) *Flasks*.—Suction flasks of 500 ml. capacity and volumetric flasks of 100–500 ml. capacity.

(c) *Funnels*.—Small Büchner funnels or sintered glass funnels of medium porosity.

(d) *Hand shears*.

(e) *Mortar and pestle*.—A deep glass mortar approximately 4" in diameter, with a well-defined lip, is recommended.

(f) *Photoelectric colorimeter*.—Calibrated for chlorophyll by means of a plant extract with light filters that give maximum light transmission near 6600 Å.

(g) *Rubber policeman*.

(h) *Wash bottles*.—The type fitted with a rubber bulb, which permits operation with one hand, is recommended.

(i) *Waring blender or similar machine*.—Vessels similar to No. 3 shown in *This Journal*, 25, 583, possess advantages over the original blender container.

(j) *Wiley mill or similar machine*.—For grinding dried material.

#### 2

##### REAGENTS

(a) *Acetone*.—Commercial undiluted acetone of technical grade and 85% aqueous solution are satisfactory.

(b) *Calcium or sodium carbonate*.—C.P. grade.

(c) *Quartz sand*.—Acid-washed and dried.

#### 3

##### DETERMINATION

Select field material carefully to insure a representative sample. Remove a representative portion from the field sample, and if fresh material cut finely with hand shears and mix as thoroughly as possible. Grind dried material in a mill and mix thoroughly. Weigh 1–5 grams into the mortar and add a small quantity (approx. 0.1 gram) of Ca or Na<sub>2</sub>CO<sub>3</sub>. Macerate the tissue with the pestle, add the quartz sand, and grind for a short time; then add the 85% acetone solution, a little at a time, and continue grinding until the tissue is finely ground. Transfer the mixture to the fun-

\* For report of Subcommittee A and action by the Association, see *This Journal*, 27, 49 (1944).

nel, draw off the solution with suction, and wash the residue with the 85% acetone solution. Return the residue to the mortar with more acetone solution and grind again. Filter, and wash as before. Repeat this procedure until the tissue is devoid of green color and the washings are colorless. (It is advisable to grind the residue at least once with undiluted acetone and then to add sufficient water at the end to bring it to 85% solution. A Waring blender or similar machine may be used for macerating and extracting the tissue instead of the mortar (see 6), but each investigator should satisfy himself that complete extraction of the tissue is accomplished by the device used.) When extraction is complete, transfer the filtered extract to a volumetric flask of appropriate size and make to volume.

Measure the percentage of light transmitted by the solution with a photoelectric colorimeter that has been calibrated for chlorophyll by use of a plant extract as described earlier in this report. Read the amount of chlorophyll present from the curve relating light transmission and concentration. Express chlorophyll values as mg./gram of tissue, or in any other convenient manner.

*Spectrophotometric Method for Total Chlorophyll and the a and b Components*<sup>7,8</sup>

## 4

## APPARATUS

The apparatus listed under 1, with the exception of a photoelectric colorimeter and a Wiley mill, plus the following:

(a) *Bottles*.—Small reagent bottles of approximately 60 ml. capacity, with either glass or cork stoppers, are convenient receptacles in which to bring the diethyl ether solutions to the proper dilution.

(b) *Pipets*.—Volumetric pipets of various capacities are required for making necessary dilutions, also straight dropping pipets (medicine droppers).

(c) *Scrubbing tubes for washing ether solutions*.—Open tube of approximately 20 mm. diameter, to one end of which is sealed a tube of smaller diameter drawn to a fine jet at the lower end.

(d) *Separatory funnels*.—250–500 ml. capacity.

(e) *Double-deck separatory funnel support*.

(f) *Spectrophotometer*.—Must be capable of isolating a spectral region of about 30 Å. near 6600 Å. with negligible stray radiation. Tubulated cells with tightly fitting glass stoppers are recommended for work with diethyl ether.

## 5

## REAGENTS

Those listed under 2 plus the following:

(a) *Diethyl ether*.—Commercial ether designated as purified for fat extraction is satisfactory without further purification.

(b) *Sodium sulfate*.—C.P. grade, anhydrous.

(c) *Trisodium phosphate*.—Glassware used in this work should be washed with a strong solution of this salt in order to remove traces of acid that might tend to decompose chlorophyll.

## 6

## DETERMINATION

(a) *Extraction of chlorophyll from tissue*.—Select and prepare the sample as directed in 3. Disintegrate a weighed portion (2–10 grams depending upon the chlorophyll content) of fresh plant tissue in a Waring blender cup, which contains a small quantity (approx. 0.1 gram) of  $\text{CaCO}_3$ , or by use of a mortar as described in 3. After the tissue is thoroughly disintegrated, filter the extract through a Büchner funnel fitted with a quantitative filter paper. Wash the residue with the 85% acetone solution, and if necessary use a little diethyl ether to remove the last traces of pigment.



If extraction is incomplete, return the residue and the filter paper to the blender container with more acetone solution and repeat the extraction. Filter, and wash as directed previously into the flask containing the first filtrate. Transfer the filtrate to a volumetric flask of appropriate size and make to volume with the 85% acetone. Pipet an aliquot of 25–50 ml. into a separatory funnel containing about 50 ml. of diethyl ether. Add distilled water carefully until it is apparent that all the fat-soluble pigments have entered the ether layer. Draw off and discard the water layer. Place the separatory funnel containing the ether solution in the upper rack of the support. Add approximately 100 ml. of distilled water to a second separatory funnel and place it in the rack below the first. Set the scrubbing tube in place and allow the ether solution to run through it to the bottom of the lower funnel and rise in small droplets through the water. When all the solution has left the upper funnel, rinse it and the scrubbing tube with a little ether added from a medicine dropper. Place the scrubbing tube in the upper funnel and exchange its place in the support with the funnel now containing the ether solution. Draw off and discard the water in the upper funnel, add a similar portion of fresh water to the lower funnel, and repeat the washing process. Continue washing the ether solution until all of the acetone is removed (5–10 washings). When washing is complete, transfer the ether solution to a 100 ml. volumetric flask, make to volume, and mix.

(b) *Spectrophotometric measurements*.—Add about a teaspoonful of anhydrous  $\text{Na}_2\text{SO}_4$  to a 60 ml. reagent bottle, and fill it with the ether solution of the pigment. When this solution is optically clear, pipet an aliquot into another dry bottle and dilute it with sufficient dry ether to cause the  $\log_{10} I_0/I$  value to fall between 0.2 and 0.8 at the wave length to be used. The most favorable value is near 0.6 at 6600 Å., since such a solution will yield a satisfactory value at 6425 Å. Fill two clean glass-stoppered absorption cells with dry ether by use of a pipet, and polish the outside surfaces of each, first with cotton wet with ethanol and then with dry cotton. Place the cells in the instrument, and determine whether each gives the same galvanometer deflection. If not, clean again or select cells that do, and follow this procedure daily. Empty one cell, fill it with the dried ether solution, and place it in the instrument. Adjust the entrance and exit slits until the spectral region isolated is 30–40 Å. at 6600 Å. Determine whether the instrument is in proper adjustment for wave length by taking readings through the solvent and the solution at intervals of 10 Å. from 6580–6650 Å. Calculate the  $\log_{10} I_0/I$  value for each wave length at which readings were taken. The highest value should occur at 6600 Å.; if it does not, adjust the machine until it does or make the 6600 Å. readings at the wave length setting that gave the highest value. In the case of a grating instrument apply the same correction at 6425 Å.; however, with a prism instrument the correction at 6425 Å. must be obtained from a wave length calibration curve for the particular instrument in use. Calibrate the instrument for wave length in this way often enough to insure that it remains in proper adjustment. Take  $I_0$  and  $I$  readings at 6600 and 6425 Å. (or the corrected settings) for each unknown solution.

(c) *Calculation of chlorophyll concentration*.—Calculate the  $\log_{10} I_0/I$  values for each of the readings made, substitute them in the following simplified equations, and solve for total chlorophyll and each of the  $a$  and  $b$  components as follows:

$$\begin{aligned} (1) \text{ Total chlorophyll (mg./liter)} &= 7.12 \log_{10} \frac{I_0}{I} \text{ (at 6600 Å.)} \\ &+ 16.8 \log_{10} \frac{I_0}{I} \text{ (at 6425 Å.).} \end{aligned}$$

$$(2) \text{ Chlorophyl } a \text{ (mg./liter)} = 9.93 \log_{10} \frac{I_0}{I} \text{ (at 6600 } \text{\AA}.) \\ -0.777 \log_{10} \frac{I_0}{I} \text{ (at 6425 } \text{\AA}.)$$

$$(3) \text{ Chlorophyl } b \text{ (mg./liter)} = 17.6 \log_{10} \frac{I_0}{I} \text{ (6425 } \text{\AA}.) \\ -2.81 \log_{10} \frac{I_0}{I} \text{ (at 6600 } \text{\AA}.)$$

### SUPPLEMENTARY INFORMATION

The factors involved in the spectrophotometric analysis of the chlorophyl system have been discussed in detail by Comar and Zscheile.<sup>6</sup> These authors used Beer's law in the form:

$$c = \frac{\log_{10} \frac{I_0}{I}}{\alpha l}, \text{ where}$$

$I_0$  is intensity of light transmitted by the solvent-filled cell,  
 $I$  is intensity of light transmitted by the solution-filled cell,  
 $c$  is concentration of chlorophyl (grams/liter),  
 $\alpha$  is specific absorption coefficient, and  
 $l$  is thickness of solution layer in cm.

Since at a given wave length the observed  $\log_{10} I_0/I$  value of a solution having two components represents the sum of the  $\log_{10} I_0/I$  values of each of the components, the following equation obtains in the case of chlorophyls  $a$  and  $b$  at a given wave length:

$$(4) \left( \log_{10} \frac{I_0}{I} \right)_{\text{observed}} = \left( \log_{10} \frac{I_0}{I} \right)_a + \left( \log_{10} \frac{I_0}{I} \right)_b.$$

If a 1 cm. cell is used this equation may be expressed as:

$$(5) \left( \log_{10} \frac{I_0}{I} \right)_{\text{observed}} = \alpha_a c_a + \alpha_b c_b.$$

The concentrations of chlorophyls  $a$  and  $b$  in a given diethyl ether solution can now be calculated by the use of equation (5) as follows:

(a) Determine  $\log_{10} I_0/I$  values for the solution at two different wave lengths (6600 and 6425  $\text{\AA}$ . have been found advantageous for this purpose).

(b) Select the proper specific absorption coefficient corresponding to the wave lengths used from Table 3.

(c) Substitute the observed  $\log_{10} I_0/I$  value and the specific absorption coefficient in equation (5) for each of the two wave lengths used as illustrated for 6600 and 6425  $\text{\AA}$ . in equations (6) and (7). Solve these two equations simultaneously for the two unknowns, the concentrations of chlorophyls  $a$  and  $b$ .

$$(6) \log_{10} \frac{I_0}{I} \text{ (at 6600 } \text{\AA}.) = 102c_a + 4.50 c_b.$$

$$(7) \log_{10} \frac{I_0}{I} \text{ (at 6425 } \text{\AA}.) = 16.3 c_a + 57.5 c_b.$$

Equations (1), (2), and (3) were derived in this way.

The criterion for the accuracy of the chlorophyll values as determined by the spectrophotometric method is the agreement between analytical results as determined from measurements at different wave lengths. It has been demonstrated by Comar and Zscheile<sup>6</sup> that measurements at 6600 and 6425 Å. are convenient for routine analysis; however, readings may be made at other wave lengths to check these values. Specific absorption coefficients for chlorophylls *a* and *b* in diethyl ether solution that may be used for this purpose are presented in Table 3.

TABLE 3.—*Absorption constants used in analysis*  
(After Comar and Zscheile<sup>6</sup>)

WAVE LENGTH Å.	SPECIFIC ABSORPTION COEFFICIENTS (FOR DIETHYL ETHER SOLUTIONS)	
	CHLOROPHYL <i>a</i>	CHLOROPHYL <i>b</i>
6600	102.	4.50
6425	16.3	57.5
6000	9.95	9.95
5810	8.05	8.05
5680	7.11	7.11
6130	15.6	8.05
5890	5.90	10.3

These values (Table 3) may be used for calculations as follows:

(a) Values for total chlorophyll and percentage composition may be calculated from absorption values at 6600 and 6425 Å. as described.

(b) Check values for total chlorophyll may be calculated from the absorption values at the intersection points 6000, 5810, and 5680 Å.

(c) Check values for percentage composition may be calculated from the absorption values for each of the points 6130 and 5890 Å. in combination with a value of total concentration as obtained from (a) or (b).

## REPORT ON IRON IN PLANTS\*

BY ERWIN J. BENNE, *Associate Referee*, and A. JOYCE SNYDER  
(Agricultural Experiment Station, East Lansing, Mich.)

Work on methods for determining iron in plant ash has been continued since publication of the 1941 report on this subject.<sup>1</sup> As stated in that report a study of certain colorimetric methods for evaluating iron was begun in the authors' laboratory with the object of finding a method more reliable and better suited to use with photoelectric colorimeters than one depending upon the color of ferric thiocyanate in acidified aqueous solutions as given in the 1940 edition of *Methods of Analysis, A.O.A.C.*

\* Published with permission of the Director of the Experiment Station as Journal Article No. 663 (n.s.)

<sup>1</sup> *This Journal*, 25, 565 (1942).

The method involving the use of *o*-phenanthroline, which forms a stable, colored complex with ferrous ions, appeared to be the most promising of the procedures investigated. Hence, the details given by Cowling in the previous report,<sup>1</sup> were submitted to interested collaborators for study. At the beginning of this study each collaborator who expressed willingness to participate was furnished three samples for analysis. Sample No. 1 was finely ground lettuce leaves, No. 2 finely ground spinach leaves,

TABLE 1.—*Results of collaborative study on evaluation of iron by o-phenanthroline and thiocyanate procedures in plant ash extracted by different methods (averages of two or more determinations expressed as mg. of Fe/gram in Samples 1 and 2 and as mg. of Fe/ml. in Sample 3)*

COLLAB-ORATOR	SAMPLE 1				SAMPLE 2				SAMPLE 3	
	O-PHENAN-THROLINE		THIOCYANATE		O-PHENAN-THROLINE		THIOCYANATE		O-PHENAN-THROLINE	THIOCYANATE
	METHOD OF EXTRACTING ASH				METHOD OF EXTRACTING ASH				EXTRACT FURNISHED	
	COWLING	A.O.A.C.	COWLING	A.O.A.C.	COWLING	A.O.A.C.	COWLING	A.O.A.C.		
1	.48	44	49	44	1 72	1 65	1.75	1 62	017	.017
(a)*	.44	.41	.47	43	1 62	1 62	1 67	1 62	017	016
(b)*	.45	42	44	43	1 65	1 65	1 63	1 65	.017	017
3	.45	.50	.53	.50	1 43	1 62	1.74	1.63	017	.015
4	.47	.41	46	42	1 63	1 51	1 75	1.71	017	019
Averages	.46	44	.48	44	1 61	1 61	1 71	1 65	.017	017

\* Obtained by use of a spectrophotometer and a visual colorimeter, respectively.

and No. 3 was an extract of spinach ash. In addition each collaborator was sent a small quantity of electrolytic iron for preparing the required iron solution, in order that a common primary standard might be used by all. The collaborators were requested to prepare extracts of the ash of portions of Samples 1 and 2 by Cowling's proposed procedure<sup>1</sup> and by the method given in *Methods of Analysis, A.O.A.C.*, 1940; and to use both the *o*-phenanthroline<sup>1</sup> and the thiocyanate methods for evaluating iron in each extract as well as in Sample 3. They were asked to use a photoelectric colorimeter for making these evaluations, if one were available, but if not, to use a visual colorimeter. If a photoelectric colorimeter was used, they were asked to state the make and to describe the light filter used. Results have been received from additional collaborators since the 1941 report was published. These results are given in Table 1.

#### COLLABORATORS AND THEIR COMMENTS

(1) *D. M. Doty and H. A. Nash, Department of Agricultural Chemistry, Purdue*

*University, Lafayette, Ind.*—The photoelectric colorimeter used was a KWSZ Photometer. A No. 430 Corning light filter, which gives maximum light transmission at 4600 Å., was used in the *o*-phenanthroline procedure; whereas, a combination of Corning light filters, Nos. 556 and GG7, which gives maximum light transmission at 5000 Å., was used with the solutions of ferric thiocyanate. In working with the thiocyanate method it was found that different lots of potassium thiocyanate gave different color intensities with solutions of identical iron contents. Hence, it was found to be necessary to establish a new standard curve for the instrument when the original supply of this reagent had been exhausted. The official A.O.A.C. method of extracting ash gave consistently lower values for iron in the referee samples and is much more tedious than the method suggested by Cowling.

(2) *J. H. Mitchell, Department of Chemistry, Clemson Agricultural College, Clemson, S. C.*—A Cenco-Sheard spectrophotometer and a Klett visual colorimeter were used for evaluating iron in different aliquots of the same solutions of the referee samples. Readings were taken on the spectrophotometer at 5000 Å. for the *o*-phenanthroline solutions and at 4700 Å. for the solutions of ferric thiocyanate. The visual colorimeter was used in the usual manner. I believe the Cowling method will give good results. I see no necessity for the porcelain crucibles. Platinum dishes should be just as good.

(3) *A. T. Perkins, C. E. Wagoner, and Douglas Chapin, Department of Chemistry, Kansas State College, Manhattan, Kan.*—The photoelectric colorimeter used was a KWSZ photometer. For the *o*-phenanthroline procedure a No. 2 WACO blue filter and 5% copper sulfate solution were used as light filters, and a No. 4 WACO green filter and 5% copper sulfate solution were used for the ferric thiocyanate solutions. We found it more difficult to make a percentage transmission vs. concentration curve for the thiocyanate procedure as the color was not stable, and it was not always possible for us to determine the percentage transmission at exactly the same time interval.

(4) *The authors.*—The photoelectric colorimeter used was a Cenco-Sheard-Sanford photometer. A No. 430, dark shade, blue-green Corning glass light filter, approximately 12.5 mm. thick (obtained by using two moulded filters of one-half this thickness), which gives maximum light transmission near 4700 Å., was used for both the *o*-phenanthroline and thiocyanate solutions. Our comments will be incorporated in the discussion.

(5) *G. D. Sherman and J. S. McHargue, Chemistry Department, University of Kentucky, Lexington, Ky.*—The results and comments of these collaborators are published in the 1941 Report;<sup>1</sup> hence, they will not be repeated here.

#### COMPARISON OF TITRIMETRIC AND COLORIMETRIC VALUES FOR IRON

Winter, in his report on plants for 1935,<sup>2</sup> gave a few data showing a comparison of results for iron by the ferric thiocyanate colorimetric and the titanous chloride titrimetric methods (*Methods of Analysis, A.O.A.C.*, 1935 and 1940). In his report for 1936<sup>3</sup> on the same subject he stated that results by these methods agree closely but gave no additional data to support the statement. He recommended that these methods be studied collaboratively; however, if such a study was undertaken the results

<sup>1</sup> *This Journal*, 19, 359 (1936).

<sup>2</sup> *Ibid.*, 20, 333 (1937).

have never been published. Hence, the authors decided to make a preliminary comparison of values by these methods and to include also the *o*-phenanthroline procedure. The values obtained are given in Table 2.

TABLE 2.—*Comparison of colorimetric and titrimetric values for iron (averages of two or more determinations expressed as mg. of Fe/ml. in the solutions and as mg. of Fe/gram in the other samples)*

SAMPLE	PROCEDURES USED—			
	O-PHENANTHROLINE	COLORIMETRIC THIOCYANATE	TITRIMETRIC TiCl <sub>3</sub>	
Standard solutions—				
No.	mg. Fe/ml.			
1	1.000	1.008	1.045	1.000
2	.500	.514	.522	.497
3	.250	.259	.256	.252
4	.125	.130	.129	.124
5	.063	.063	.065	.062
6	.031	.033	.033	.030
7	.016	.017	.018	.015
Referee samples*—				
No. 1		.46	.45	.44
No. 2		1.62	1.76	1.60
No. 3—Analyst A		.017	.018	.019
No. 3—Analyst B		.017	.017	.017
Hay*—				
Alfalfa		.013	.014	.014
Alfalfa-brome		.011	.012	.012
Brome		.012	.013	.015
Oat leaves*—				
No. 1		.018	—	.020
No. 2		.020	—	.021

\* Aliquots of the same ash extract were used with the different procedures.

## DISCUSSION

It is evident from Table 1 that each procedure used by the different collaborators gave values in good agreement for iron in the collaborative samples. Indeed the variation among results for a given sample is but little more than might be expected among a series of replicate determinations by one analyst. Moreover, values obtained by the two colorimetric procedures agree closely. As stated in the previous report,<sup>1</sup> however, it is the authors' experience that the thiocyanate procedure is less reliable than the *o*-phenanthroline procedure, since ferric thiocyanate in acidified aqueous solutions is unstable and the procedure based upon the color of this com-

bination frequently gives erratic results for iron. For example, in attempting to calibrate a photoelectric colorimeter with solutions of ferric thiocyanate, prepared according to the A.O.A.C. procedure, the authors experienced difficulty in reproducing a curve relating light transmission and concentration of iron with solutions of the same iron content. Several series of solutions, each containing the same quantities of iron, were carried through the procedure before two curves that coincided were obtained. On the other hand, calibration curves for solutions containing similar quantities of iron in the form of the *o*-phenanthroline complex could be reproduced consistently. Once the calibration curves were established, however, the two procedures gave comparable values for iron. This appears to have been the experience of the other collaborators. The authors have obtained similarly inconsistent results with the thiocyanate procedure for iron in unknown solutions for reasons that are not obvious. Although the *o*-phenanthroline procedure requires more reagents than the thiocyanate procedure, and thereby lacks some of the simplicity of the latter, the authors have found it more reliable and better suited to use with a photoelectric colorimeter than the present A.O.A.C. procedure.

With regard to the different methods of extracting ash, it will be noted that Collaborators 1 and 4 obtained results for iron by Cowling's method that are consistently higher than those by the A.O.A.C. method. This tendency is apparent also in most of the results of Collaborator 2, but it does not exist in those from the other collaborators. Such an increase could be due to liberation of iron from combinations formed during the ashing process, or as suggested by Sherman and McHargue<sup>1</sup> from sand contained in the sample, by action of the hydrofluoric acid used in Cowling's method. These inconsistencies indicate the need for further study on the extraction of plant ash for analysis of iron.

As shown by the data in Table 2 the titanous chloride titrimetric method gave values for iron in surprisingly close agreement with those obtained by the colorimetric procedures. This was true not only for the standard solutions used but for extracts of the ash from several different tissues as well. The titanous chloride method is simple and convenient, its only objectionable feature being the instability of the reagent, which makes frequent standardizations of the solution necessary to insure accurate analytical values. The ease with which this can be accomplished, however, minimizes even this objection. According to the authors' experience titanous chloride decomposes at an accelerated rate in very dilute solutions; hence, it was found to be inadvisable to titrate with a solution containing less titanium trichloride per ml. than that equivalent to .2 mg. of iron. This limits the use of this method for extremely small quantities of iron, since inaccuracies are likely to result from titration when too small a volume of solution is required. Although the authors have been able to

reproduce results consistently by the titrimetric method with aliquots containing only .1 mg. of iron, for samples containing less than this amount it would probably be advisable to use a colorimetric procedure.

Attention should be called to an error in the 1941 report concerning the concentration of the bromophenol blue solution used as an indicator in the *o*-phenanthroline procedure. The directions in the original manuscript were to prepare a .04 per cent solution by grinding .1 gram of solid bromophenol blue in a mortar with 3 ml. of 0.05 *N* sodium hydroxide solution, transferring to a volumetric flask, and diluting to 250 ml. with distilled water. Unfortunately during the printing of the article (*This Journal*, 25, 563 (f)) .04 per cent was changed to .4 per cent and .1 gram to 1 gram. Anyone desiring to use the method should of course use the correct figures.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the thiocyanate method for the colorimetric evaluation of iron given in *Methods of Analysis*, A.O.A.C., 1940, be dropped.

(2) That the study of the *o*-phenanthroline procedure for the colorimetric, and the titanous chloride method for the titrimetric, evaluation of iron be continued with the view to adopting them as official methods of the A.O.A.C.

(3) That further study be given to the ashing of plant material and extraction of the ash for the analysis of iron.

#### ACKNOWLEDGMENT

The authors sincerely thank the collaborators for their cooperation in this work.

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### REPORT ON SOILS AND LIMING MATERIALS

By W. H. MACINTIRE (The University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Referee*

Because of changes and losses of personnel and the subordination of all problems to the demands of the War Program, the only collaborative work done during the past year has been studies upon CO<sub>2</sub> determinations and upon the factors that affect the titrimetric determination of the neutralizing values of slags of variant type and composition. These studies have been conducted in the laboratories of the Referee as integrated phases of established investigations. The results of the CO<sub>2</sub> study appeared in a recent issue of *This Journal*, "Steam Distillation—A New Procedure for the Determination of Carbonate CO<sub>2</sub>" (August 1943). The

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 27, 49 (1944).



studies as to titrative procedures for the evaluation of slags have resulted in a procedure that is set forth in the report of the Associate Referee on Liming Materials. A detailed report on the studies of the several factors that influence the dissolution and titration of slags will be given in a paper to be tendered *This Journal*.

It is hoped that circumstances will admit of further work on the determination of boron during the coming year.

It is recommended\*—

(1) That the proposed method for the titrative evaluation of slags be adopted as tentative and studied further.

(2) That collaboration be sought in furtherance of the several contemplated studies.

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## REPORT ON LIMING MATERIALS

By W. M. SHAW (The University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*

The following recommendations are made upon the basis of experimental data that will be presented in a separate paper to be tendered *This Journal* in the near future.

It is recommended\* that the following procedure be adopted as tentative for the determination of the neutralizing value of calcium silicate slags:

### NEUTRALIZING VALUE OF CALCIUM SILICATE SLAG

(a) *Blast Furnace Slags*.—Weigh .5 gram charge ground to pass 80-mesh sieve and transfer into a 250 ml. Erlenmeyer flask. Wash down with small portions of water and introduce 35 ml. of .5 *N* HCl while swirling. Heat to a gentle boil over Bunsen burner, *agitating the suspension continuously* until the bulk of the sample has dissolved. Maintain the boiling for 5 minutes; cool to room temperature, transfer to 150 ml. beaker, and dilute to about 80 ml. Titrate with .5 *N* NaOH to pH 4.8 as determined by a glass electrode. Net ml. of acid used times 5 = neutralizing value of slag in terms of CaCO<sub>3</sub>.

(b) *Slags from Rock Phosphate Reduction Furnaces*.—Weigh .5 gram charge and transfer into a 250 ml. beaker. Wash down with small portions of water and introduce 50 ml. of acetic acid (1+4). Stir the suspension continuously during the addition of the acid. Heat to boiling 5 minutes, stirring frequently. Evaporate to a gel on hot plate or sand bath. Add 40 ml. of the acetic acid, dilute to 150 ml., and heat to boiling; add NH<sub>4</sub>OH (1+1) to the clear yellow of methyl red and a distinct odor of ammonia. Digest about 15 minutes on hot plate. Filter by gravity on a 9 cm. paper, catching the filtrate in a 220 ml. shallow-form porcelain dish; wash beaker 3 times and the filter 5 additional times with neutral .5 *N* ammonium acetate. Evaporate filtrate on hot plate. To prevent spattering adjust heat so that the bubbles that break through the viscous surface film are released gently. (Dehydration may be expedited

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 27, 49 (1944).

by 2 or 3 repeated treatments with 25 ml. of hot water and evaporation.) Continue the heating of the residue on the heat plate until odor of acetic acid can not be detected. Heat 10 additional minutes at the full heat of the hot plate and then ignite 10 minutes in electric furnace at 550°C. Cool, wet the residue with 15 ml. of water, place cover-glass over dish, and introduce 25 ml. of .5 *N* HCl through the lip of the beaker. Heat 5 minutes over Bunsen burner to a gentle simmer. Rinse the cover-glass; filter the suspended manganese oxides on a 9 cm. filter, catching the filtrate in a 250 ml. Erlenmeyer flask; and wash dish and the filter 3 times with hot water. Titrate excess acid with .5 *N* NaOH to the clear yellow of methyl red. Net acid used  $\times 5$  = neutralizing value of the slag in terms of  $\text{CaCO}_3$ .

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## REPORT ON VITAMINS

By E. M. NELSON (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

*Vitamin A*.—There will be no report. J. B. Wilkie is the associate referee.

*Vitamin B<sub>1</sub>*.—O. L. Kline is the associate referee. The referee approves of the recommendation of the associate referee that the thiochrome method proposed be adopted as a tentative method.

*Vitamin C*.—Otto A. Bessey is the associate referee. The referee approves the recommendation of the associate referee that the proposed method be adopted as a tentative method.

*Vitamin D, Milk*.—There will be no report. W. C. Russell is the associate referee.

*Vitamin D, Poultry*.—There will be no report. Chester D. Tolle is the associate referee.

*Vitamin K*.—There will be no report. H. J. Almquist is the associate referee.

*Riboflavin*.—A. R. Kemmerer is the associate referee. The first two recommendations of the associate referee, which read as follows, are approved:

(1) That fluorometric methods for riboflavin be further studied so that such a method may be recommended for materials other than yeast, dried skim milk, and alfalfa.

(2) That the microbiological method be further studied with a view to improving the basal medium.

It is further recommended that the associate referee be instructed to study the microbiological method with a view to making the procedure identical with the procedure that has been adopted by the U.S. Pharmacopoeia.

*Nicotinic Acid*.—Harris Isbell is the associate referee. The recommendation of the associate referee that the method proposed be adopted as a tentative method is approved.

*Carotene and Cryptoxanthin in Yellow Corn.*—There will be no report. A. R. Kemmerer is the associate referee.

*Crude and Pure Carotene.*—A. R. Kemmerer is the associate referee. The recommendations of the associate referee are approved.

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## REPORT ON VITAMIN B<sub>1</sub>

By O. L. KLINE (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

It has been two years since the last report of the Associate Referee on Methods for Determination of Vitamin B<sub>1</sub> or Thiamine. In the interim considerable progress has been made in modification and adaptation of the thiochrome procedure for this determination.

In November, 1941, the Associate Referee discussed briefly before this group a survey of chemical methods for the estimation of vitamin B<sub>1</sub>. That discussion included a description of the extensive collaborative study of methods for the B vitamins applicable to cereal products that had been sponsored by the Research Corporation. At that meeting the Referee on Vitamins gave instructions to make a study of the chemical methods for thiamine and their applicability to A.O.A.C. problems. Since that time a number of studies, collaborative in nature, have been conducted by groups of workers when problems concerning specific types of food products arose. The laboratory with which the Associate Referee is associated has taken part in a number of such studies, which include additional work on the Research Corporation committees, a study sponsored by the American Association of Cereal Chemists, work in connection with surveys conducted by experiment station groups, collaborative work on methods included in the elaborate vitamin survey now being conducted by the National Canners Association, and more recently those studies carried out by the Committee on Food Composition of the National Research Council. At present results of these studies for the most part are unpublished, but all have added to the experience and knowledge of how to adapt the thiochrome method to the examination of food products.

Important progress has been made also in the application of methods for the B vitamins to pharmaceutical preparations. Proposed procedures for thiamine, riboflavin, niacin, and choline have been accepted for inclusion in the First Bound Supplement of U.S.P. XII. In conjunction with this step a well planned and extensive collaborative study of these methods has been carried out with more than 40 laboratories taking part. Although the thiochrome procedure used in the study was designed specifically for dried yeast, it has been found equally applicable to all phar-

maceutical preparations thus far examined. In a number of laboratories it has been satisfactorily applied as well to cereal products and to vegetable products. A brief description of the U.S.P. collaborative study, and the results obtained may be desirable at this time to serve as a basis for the following recommendation: "That the U.S.P. method for the determination of thiamine by the thiochrome procedure be adopted as a tentative chemical method for the examination of cereal products and vegetable products."

In June of this year Dr. E. Fullerton Cook, Chairman of the Committee on Revision of the United States Pharmacopoeia, issued an invitation to participate in a study on the proposed methods that have been mentioned, an invitation to which nearly 60 laboratories responded. Samples of dried yeast, liver B vitamins concentrate, and liver B vitamins injection and U.S.P. Reference Standards for thiamine, riboflavin, and niacin were distributed. Descriptions of the methods were supplied, and assayers were asked to follow them in exact detail; 41 laboratories reported results on one or more of the samples, and the data from 39 of these have been tabulated and summarized. Two reports arrived after the tabulation had been completed. Mimeographed tables on the data and descriptions of the methods used have been distributed. The summary of the data relating to thiamine presented here includes the average of the values obtained by the proposed U.S.P. procedures, the number and proportion of those falling within  $\pm 10$  per cent of the average value; the range, that is, the high and low value; and the number of values falling more than 10 per cent below and more than 10 per cent above the average value. For the results of "other" methods only the average is given.

The data on dried yeast in the table include the results from 39 laboratories; 33 of these reported a value for thiamine by the proposed U.S.P. method. Of the 33 values 25 (or 76 per cent) fall within  $\pm 10$  per cent of the average value of 276. In view of the widely varied experience of assayers in a group as large as this the agreement may be considered remarkably good. It is clear, however, from comments made by the collaborators that the result is somewhat below the true thiamine value for this sample of dried yeast. Owing to faulty wording in the description of the method as submitted to the collaborators, many of the assayers failed to carry the standard thiamine solution through all the steps that are used in preparation of the assay solution. This is important because small differences in technic, differences in adsorption capacity of the base exchange material from various sources, and differences in degree of recovery of the thiamine from the base exchange column may affect the final result. Results given under "other" methods for thiamine include a value obtained by the U.S.P. rat-curative technic, and one obtained by the fermentation procedure. These values, 300 and 312, are in close agreement with a result

TABLE 1.—Results of U.S.P. collaborative study of dried yeast  
(Expressed as microgram/gram.)

	THIAMINE		RIBOFLAVIN		NIACIN	
	PROP. U.S.P. METHOD	OTHER METHODS	PROP. U.S.P. METHOD	OTHER METHODS	PROP. U.S.P. METHOD	OTHER METHODS
1	300			130		800
2	241		54		530	
3	280		58	59	550	547
4	271	278	56		546	
5	280		76		590	
6	282		62	63		
7						517
8	245	307	60		540	
9	274		61		522	
10			55			
11	303		78		511	
12	263		40		560	
13	292	291	50		515	
14			68	64	580	
15	305		57		534	
16	310		65		660	
17	252		61		623	
18	265		62		683	
19	163		53			
20	280			61		388
21			59		596	
22	206		70		583	
23				71		508
24	276	298		65		501
25	210		53		604	
26	365		58		500	
27	249			41		
28	309	316		66		611
29	276		62		528	
30	269		64	67		
31	293		49		550	
32	286		45		503	
33	286	279		54		448
34			100			
35	290		72	71	487	
36	284		60		547	
37	300		62	56		
38	291		64		576	
39	305	300	62		580	
		312				
Av.	(33) 276	(8) 299	(31) 61	(13) 67	(25) 560	(8) 540
High	365		— 100		683	
Low	163		— 40		489	

25 (76%) fall between  
248 and 305 ( $\pm 10\%$  of  
276)

3 above 305  
5 below 248

17 (57%) fall between  
55 and 67 ( $\pm 10\%$  of  
61)

6 above 67  
7 below 55

19 (76%) fall between  
504 and 616 ( $\pm 10\%$  of  
560)

3 above 616  
3 below 504

of 305 determined by the thiochrome procedure in which the standard solution and assay solution were treated alike. As a result of this study and the comments received from the collaborators changes have been made in the description of the method, and it is hoped that they will prevent further misinterpretation.

#### RECOMMENDATIONS\*

It is recommended—

(1) That the U.S.P. method for the determination of thiamine by the thiochrome procedure be adopted as a tentative chemical method to be applied to cereal products and vegetable products.

(2) That during the next year modifications of this technic be studied to determine a suitable treatment for other foods such as meat products.

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### REPORT ON ASCORBIC ACID (VITAMIN C) IN CITRUS FRUITS AND TOMATOES

By OTTO A. BESSEY (The Public Health Research Institute  
of The City of New York, Inc.), *Associate Referee*

Ascorbic acid (vitamin C) reduces, stoichiometrically and rapidly, a number of substances, e.g., certain metal ions, iodine, iodate, and certain oxidation-reduction indicators. This is the most characteristic chemical property of the vitamin and has, therefore, served as a basis for a number of analytical methods, the most practical of which is the indophenol method.<sup>1</sup> While this type of reaction is by no means specific, it has been shown that by selection of the proper conditions it can be used as a practical and reliable measure of ascorbic acid in certain products.

Reducing substances other than ascorbic acid may occur in some foods or be developed during processing, e.g., heating alkaline sugar solution, baking, dehydration, etc. In these cases, analytical methods based on oxidation-reduction reactions are obviously unsatisfactory. Also such methods do not measure the oxidized (dehydroascorbic acid) but still biologically active form of ascorbic acid which may develop in some products under certain conditions.

After several years' experience in many laboratories there is no evidence that dehydroascorbic acid or other reducing substances than ascorbic acid occur in fresh or commercially canned citrus fruits or tomatoes. Furthermore, comparison of the indophenol method with the independent (furfural) method of Roe<sup>2</sup> as applied to these products gives satisfactory checks. In view of these experiences and observations it seems reasonably

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 27, 48 (1944).

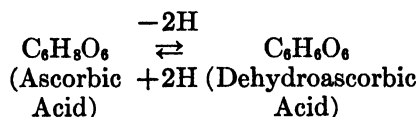
<sup>1</sup> Bessey, Otto A., *J. Am. Med. Assoc.*, 111, 1290-98 (1938).

<sup>2</sup> *J. Biol. Chem.*, 116, 609-619 (1936).

certain that the indophenol method when used as described below does in fact measure the ascorbic acid value of citrus fruits and tomato juice. The method has been extensively used with satisfaction by the Food and Drug Administration, the Food Distribution Administration, and by many other laboratories, including that of the Associate Referee, and therefore it seems suitable for recommendation as a tentative method for the A.O.A.C. It is planned, now that methods for more thoroughly testing the reliability of the proposed method are available, to organize such studies for the coming year.

The indophenol method recommended follows essentially that of Bessey and King,<sup>3</sup> which was later modified by Musulin and King<sup>4</sup> and Bessey.<sup>5</sup> One may obtain more detailed information concerning the chemistry and underlying principles upon which the analysis is based by consulting the publications of King,<sup>6</sup> Harris and Ray,<sup>7</sup> Bessey,<sup>1</sup> Kon and Watson,<sup>8</sup> Barron,<sup>9</sup> Fujita and Ebihara,<sup>10</sup> and Harris and Olliver.<sup>11</sup>

Briefly, the basic principles of the method depend upon two characteristics of ascorbic acid; namely, its relative stability in acid solutions and its ability to reduce rapidly to a colorless solution the highly tinctorial oxidation-reduction indicator dye, 2, 6-dichlorophenol indophenol. The dye, although not specific for ascorbic acid, has an active oxidation-reduction range nearer to the ascorbic-dehydroascorbic acid system than have some of the other suggested oxidizing reagents, e.g., iodine or iodate. The reaction is run in the presence of acetic acid and metaphosphoric acid. These reagents serve a four-fold purpose: first, to maintain the necessary acidity; second, to inhibit oxidation catalyzed by certain metallic ions; third, to inactivate enzymes; and fourth, to precipitate proteins and liberate protein-bound ascorbic acid. The chemical reaction is—



The oxidation of one mol of ascorbic acid requires one mol of the dye. It will be noted that the reaction is a reversible one in which two hydrogen equivalents are involved. Ordinarily dehydroascorbic acid has not been found to exist in plant materials. Some exceptions reported are potatoes and maturing apples. A special technic necessary in the assay of the oxidized form requires the use of hydrogen sulfide to reduce the dehydroas-

<sup>3</sup> *J. Biol. Chem.*, 103, 687-698 (1933).

<sup>4</sup> *Ibid.*, 116, 409-413 (1936).

<sup>5</sup> *Ibid.*, 126, 771-784 (1938).

<sup>6</sup> *Physiol. Rev.*, 16, 238-262 (1936).

<sup>7</sup> *Biochem. J.*, 27, 580-589 (1933).

<sup>8</sup> *Ibid.*, 30, 2273-2290 (1936).

<sup>9</sup> *J. Biol. Chem.*, 112, 625-640 (1936); 116, 563-573 (1936).

<sup>10</sup> *Biochem. Z.*, 301, 229-237 (1939).

<sup>11</sup> *Biochem. J.*, 36, 155-182 (1942).

corbic acid. Information covering a reduction procedure and analysis involving dehydroascorbic acid is given by Bessey<sup>5</sup>; however, when dealing with the types of food products mentioned herein this phase of the problem need not be considered.

ASCORBIC ACID (VITAMIN C) APPLICABLE TO ORANGE, GRAPEFRUIT, LEMON,  
LIME, AND TOMATO JUICE

## 1

## REAGENTS

(a) *Metaphosphoric acid-acetic acid stabilizing extracting solution*.—Dissolve with shaking 15 grams of stick glacial  $\text{HPO}_3$  in 40 ml. of glacial acetic acid and 200 ml. of distilled water, dilute to approximately 500 ml., and filter rapidly through a fluted filter into a glass-stoppered bottle. (The  $\text{HPO}_3$  slowly changes to  $\text{H}_3\text{PO}_4$ , but if stored in refrigerator, it remains satisfactory for 7–10 days.)

(b) *Sodium bicarbonate*.—0.05 *M*  $\text{NaHCO}_3$ . Dilute 10 ml. of  $\text{NaHCO}_3$  with 40 ml. of water.

(c) *Sodium 2,6-dichlorobenzeneoneindophenol (2,6-dichlorophenolindophenol, sodium salt) solution*.—Dissolve 0.05 gram of reagent-grade indophenol dye, which has been stored in a desiccator over soda-lime, in 50 ml. of water to which has been added 42 mg. of  $\text{NaHCO}_3$ ; shake vigorously, and when the dye has dissolved dilute to 200 ml. with water. Filter through a fluted filter into an amber glass-stoppered bottle. Keep stoppered, out of direct sunlight, and store in refrigerator. (Decomposition products that make the end point indistinct occur in some batches of dry indophenol and also develop with time in the stock solution. Add 5.0 ml. of extracting agent containing excess ascorbic acid to 15 ml. of the dye reagent. If the reduced solution is not practically colorless, discard and prepare a new stock solution. If the dry dye is at fault, obtain a new specimen.)

(d) *Standard ascorbic acid*.—Use U.S.P. Reference 1-ascorbic acid (obtainable from Dr. E. Fullerton Cook, 43rd St. and Woodland Ave., Philadelphia, Penn.). Keep cool, dry, and out of light.

(e) *Indophenol*.—Weigh accurately ( $\pm 0.1$  mg.) approximately 0.1 gram of the U.S.P. Reference 1-ascorbic acid, transfer to 100 ml. glass-stoppered volumetric flask, and bring to mark (room temp.) with the metaphosphoric acid-acetic acid reagent. Standardize the indophenol reagent at once as follows: Transfer three 2.0 ml. aliquots of the standard to each of three 50 ml. Erlenmeyer flasks containing 5.0 ml. of the metaphosphoric acid-acetic acid reagent. Titrate rapidly with the indophenol reagent from a 50 ml. buret until there persists a light but distinct rose-pink color for at least 5 seconds. (Each of the titrations should require about 15 ml. of the indophenol reagent and they should check well within 0.1 ml.) In like manner titrate three blanks composed of 7.0 ml. of the metaphosphoric acid-acetic acid reagent plus a volume of water approximately equivalent to the volume of indophenol reagent used in the direct titrations. After subtracting the average blanks (usually about 0.1 ml.) from the standardization titrations, calculate and express the strength of the indophenol reagent as mg. of ascorbic acid equivalent per 1.0 ml. of reagent. Standardize the indophenol reagent each day of use with a freshly prepared standard ascorbic acid solution.

## 2

## PREPARATION OF SAMPLE AND DETERMINATION

Prepare juices as directed under XXVI, 2, p. 335, *Methods of Analysis, A.O.A.C.*, 1940. Add aliquots of at least 100 ml. of the prepared juice to equal volumes of the metaphosphoric acid-acetic acid reagent. Mix, and filter. Titrate 10 ml. aliquots as directed for standardization of the indophenol reagent, and make blank determina-



tions for corrections of the titrations as described under 1(e), using the proper volumes of acid reagent and water. Express ascorbic acid as mg./100 ml. of original juices.

NOTE: Products containing ferrous iron, originating from defective cans or otherwise, give values in excess of their actual ascorbic acid content by this method. The following is a simple test to ascertain whether ferrous iron is present to an extent that invalidates the test.—Add 2 drops of 0.05% water solution of methylene blue to 10 ml. of the freshly prepared sample of juice and the metaphosphoric acid-acetic acid reagent. A disappearance of the methylene blue color after 5–10 seconds of mixing indicates the presence of interfering substances.

The Associate Referee wishes to acknowledge the fact that the details of the methods as described above are essentially those written and used by W. L. Hall of the Food and Drug Administration, Washington, D. C.

## REPORT ON RIBOFLAVIN

By A. R. KEMMERER (Agricultural Experiment Station,  
College Station, Texas), *Associate Referee*

Fluorometric and microbiological methods for riboflavin in yeast and dried skim milk, *This Journal*, 24, 413, and for alfalfa, *Ibid.*, 25, 101, 459, have been tentatively adopted. In last year's report, *Ibid.*, 26, 81, a recommendation was made that a modified microbiological technic, given below in the recommendations, be tentatively adopted for whole wheat and white flour. This recommendation was based on the results of a collaborative study which are given in Tables 1 and 2, *Ibid.*, 84, 86, of last year's

TABLE 1.—*Effect of filtration at pH 4.5 on riboflavin assay*

SAMPLE	RIBOFLAVIN		DIFFERENCE FROM ORIGINAL METHOD
	ORIGINAL METHOD	FILTERED, pH 4.5	
	p.p.m.	p.p.m.	per cent
Alfalfa	16.4	14.9	9.2
Wheat gray shorts	5.7	3.4	40.4
Fish meal	9.9	5.0	49.5
Soy bean oil meal	6.2	2.8	54.9
Whole wheat flour	1.8	0.9	50.0
White flour	0.7	0.4	42.9

report. In this modified method the acid extracts of the test materials are adjusted to pH of 4.5 and filtered. This procedure removes materials that otherwise would cause high results by stimulating the production of excess acid by *Lactobacillus casei*. Recent work by Strong and Carpenter<sup>1</sup> and

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 14, 909, (1942).

by Wegner, Kemmerer, and Fraps<sup>2</sup> shows that the modified technic gives better results than does the original method on other materials as well as wheat flours. Representative data obtained by the latter workers<sup>2</sup> are given in Table 1. The precipitates removed did not contain riboflavin but enhanced the action of added riboflavin.

Landy and Dicken<sup>3</sup> and Barton-Wright<sup>4</sup> claim that the microbiological method can be improved by the use of a modified basal medium. Studies on changes in the medium should be conducted.

In the report presented here it is the purpose of the Associate Referee to restate the recommendation made last year and to make recommendations that will incorporate recent improvements in the microbiological methods already tentatively adopted. These recommendations are also made with the view to the adoption of one general method that can be used for practically all materials.

#### RECOMMENDATIONS\*

It is recommended—

(1) That fluorometric methods for riboflavin be further studied so that such a method may be recommended for materials other than yeast, dried skim milk, and alfalfa.

(2) That the microbiological method be further studied with a view to improving the basal medium.

(3) That the following microbiological method, *This Journal*, 26, 81, Tables 1 and 2, be tentatively adopted for the determination of riboflavin in foods and feeds and in place of the microbiological methods tentatively adopted previously for yeast and dried skim milk, *Ibid.*, 24, 416, and alfalfa, *Ibid.*, 25, 101.

*Reagents.*—Proceed as directed in *This Journal*, 24, 417–18, except to provide that the pure culture specified as *Lactobacillus casei* e be termed by its correct name, *Lactobacillus casei*.

*Preparation of samples.*—Weigh 2–10 grams of the sample to be assayed, depending on the riboflavin content (yeast 2 grams, alfalfa and dried skim milk 3 grams, and wheat flours 10 grams). Add 140 ml. of 0.1 N HCl. Mix well and autoclave 15 minutes at 15 lbs. pressure. (If the sample contains over 5 p.p.m. of riboflavin and there is any appreciable amount of material not dissolved by the autoclaving, centrifuge off the insoluble material and to it add 140 ml. 0.1 N HCl and autoclave again for 15 minutes at 15 lbs. pressure. Combine with the first extract (do not remove insoluble material). Cool to room temperature. By means of a pH apparatus with a glass electrode adjust the pH to 4.5 with normal NaOH. Filter the sample carefully, pouring the first few ml. that come through back onto the filter paper. Wash the precipitate two times with 10 ml. portions of distilled water, buffered with 1 ml. phosphate solution, *This Journal*, 24, 417 (e), and adjusted to pH 4.5 with 0.1 N HCl. Adjust the pH to 6.6–6.8 and dilute to such a volume that the solution contains 0.05–0.1 p.p.m. of riboflavin.

<sup>2</sup> *J. Biol. Chem.*, 146, 547, (1942).

<sup>3</sup> *J. Lab. Clin. Med.* 27, 1086 (1942).

<sup>4</sup> *Biochem. J.*, 37, 25 (1943).

\* For report of Subcommittee A and action by the Association, see *This Journal*, 27, 48 (1944).

**Determination.**—With each set of assays run known levels of riboflavin consisting of 0.0, 0.5, .10, .15, and .20 microgram of riboflavin. Put 4 tubes on each level. With a semi microburet or semi micropipet measure 0.0, 0.5, 1.5, and 2.0 ml. of the Standard Riboflavin Solution C, *This Journal*, 24, 417, into test tubes and pipet in 5 ml. of the liquid culture medium. Add enough water to make the final volume 10 ml.

Assay the test solutions at 2 or more levels which contain 0.05–0.20 micrograms of riboflavin. Place 4 tubes on each level. With a semi microburet or semi micropipet measure 0.5–5.0 ml. of test solution into test tubes and pipet in 5 ml. of liquid culture medium. Add enough distilled water to make the final volume 10 ml.

Plug the tubes with non-absorbent cotton, sterilize in the autoclave at 15 lbs. pressure for 15 minutes, allow to cool, and inoculate each with 1 drop of the 0.9% NaCl inoculum. Incubate at 37°C. for 3 days. Transfer contents to 125 ml. Erlenmeyer flasks, wash the tubes with 10–20 ml. of distilled water, and titrate to pH of 7.0 with 0.1 N NaOH, using bromothymol blue as indicator.

**Calculation of results.**—Proceed as directed in *This Journal*, 24, 419.

## REPORT ON CAROTENE IN FEEDING STUFFS

By A. R. KEMMERER (Texas Agricultural Experiment Station,  
College Station, Texas), *Associate Referee*

In the last two reports of the Associate Referee (*This Journal*, 25, 886; 26, 77), the need of a complete chromatographic method for the chemical estimation of biologically active carotenoids was pointed out. This conclusion has been amplified by the recent findings of several laboratories. Beadle and Zscheile<sup>1</sup> have shown that fresh vegetables contain appreciable amounts of neobeta carotene, an isomer of beta carotene that has less biological activity than has beta carotene. Polgar and Zechmeister<sup>2</sup> have pointed out that beta carotene isomerizes into a number of cis-trans isomers. Kemmerer and Fraps<sup>3</sup> in recent work have shown that crude carotene extracts of plant materials may contain, besides the neo-beta-carotene of Beadle and Zscheile, appreciable amounts of biologically inactive pigments which they term impurity A and carotenoid X. Representative data from the latter work are given in Table 1.<sup>3</sup> The only way in which these pigments could be separated was by chromatographic analysis on calcium hydroxide. No existing method for pure carotene that was tried would remove neobeta carotene and carotenoid X although such methods, including the method for pure carotene, tentatively adopted (*This Journal*, 25, 891), removed impurity A to a large extent. Magnesium oxide, as recommended in the previous reports for chromatographic analyses, does not separate these isomers. Therefore, when the constituents of a carotene solution or of a crude carotene fraction are desired the complete chromatographic method utilizing calcium hydroxide is needed. That reliable

<sup>1</sup> *J. Biol. Chem.*, 144, 21, (1942).

<sup>2</sup> *J. Am. Chem. Soc.*, 64, 1856 (1942).

<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 15, 714 (1943).

TABLE 1.—*Constituents of crude carotene in dehydrated alfalfa leaf meal*

SAMPLE NO.	CRUDE CAROTENE	IMPURITY A	CONSTITUENTS (PER CENT)		NEOBETA CAROTENE
			CAROTENOID X	BETA CAROTENE	
	<i>p.p.m.</i>				
1	23.5	27.0	—	66.4	6.6
2	172.5	9.4	12.6	67.9	10.1
3	102.5	29.7	26.7	26.4	17.2
4	188.3	5.8	23.1	53.3	17.8
5	172	12.7	20.5	50.2	16.6

results can be obtained by a similar method is shown by the results of the collaborative study carried out last year (*This Journal*, 26, 80, Table 3). The substitution of calcium hydroxide for magnesium oxide causes no change in the procedure.

The method adopted by the A.O.A.C. for crude carotene applies only to dried hays and plants. With slight modifications this method can be extended to other materials. Such methods are described in the recommendations.

In some laboratories it is difficult to obtain the ethyl alcohol specified in the A.O.A.C. method. A method that does not require alcohol and that has given reliable results for the Associate Referee is as follows:

To 1 or 2 grams of finely ground alfalfa meal add 100 ml. of petroleum benzine containing 5% acetone and allow the mixture to stand overnight. Filter on a sintered glass funnel and wash the residue 3 times with 15 ml. portions of the solvent. Shake

TABLE 2.—*Pure carotene in alfalfa by A.O.A.C. method and the method without alcohol*

SAMPLE NO.	A. O. A. C. METHOD	PURE CAROTENE	
			METHOD WITHOUT ALCOHOL
	<i>p.p.m.</i>		<i>p.p.m.</i>
1	78		75
2	44		40
3	51		50
4	59		55
5	72		72
6	250		250
7	57		56
8	180		178
9	21		20

the petroleum benzine extract four times with water to remove the acetone, and dry with anhydrous  $\text{Na}_2\text{SO}_4$ . Dilute to 200 ml. or some other appropriate volume. Evaporate a 50 ml. aliquot in vacuo to approximately 10 ml. and pass through a column

of  $\text{MgCO}_3$  as specified in the method recommended below for tomatoes and water melons. Wash the column until all the carotene is washed through. Dilute the portion that comes through the column to appropriate volume and determine the carotene by one of the A. O. A. C. methods (*Methods of Analysis*, A. O. A. C., 1940, 369).

A few results obtained by this method are compared in Table 2 with results obtained by the tentatively adopted method for pure carotene. (*This Journal*, 25, 891).

#### RECOMMENDATIONS\*

It is recommended—

(1) That the method for crude carotene (*Methods of Analyses*, A.O.A.C. 1940, 369) be extended to materials other than dried hays and dried plants. The required modifications follow.

(a) *Fresh green materials*.—For samples to be analyzed soon after gathering (1–2 hours), place 100 grams of material in a large evaporating dish, soak in 100 ml. of 95% ethyl alcohol for 5 minutes, and cut up with scissors. Add 100 grams of clean white sand, free of organic matter, and grind until a uniform mixture is obtained. For samples necessitating shipment or delay in analysis, place approximately 100 grams of material in a tared fruit jar with a weighed quantity of alcohol or methanol. Seal the jar by using a jar rubber under lid. Upon arrival at laboratory, weigh jar and contents and subtract weight of jar and alcohol from total weight to obtain weight of sample.

Pour contents of jar into an evaporating dish, cut up with scissors, and grind with sand as directed above. Decant liquid from solid part through a cheese cloth and make up to volume. Weigh solids. Take aliquots of both solid and liquid, equivalent to 5 grams of the fresh untreated material and mix together for carotene analysis. Saponify the sample by boiling for 30 minutes in 50 ml. of 12% alcoholic KOH. Cool, add 50 ml. of petroleum benzine, and decant liquid into a separatory funnel. Transfer residue to a mortar and grind with pestle, first with a 15 ml. portion of petroleum benzine and then with a mixture of 5 ml. of 95% ethyl alcohol and 15 ml. of petroleum benzine until no further color is extracted. Then proceed as directed in 61, p. 369, *Methods of Analysis*, A. O. A. C., 1940.

(b) *Fresh green materials that have been preserved in methanol or ethyl alcohol and where only crude carotene on dry bases is desired*.—Pour off as much of the alcohol as possible. Grind the solid portion in a food chopper and place in a tightly sealed jar to prevent evaporation. Weigh out 5 grams of the solid material and proceed as directed in (a). Determine dry matter in a portion of the sample.

(c) *Fresh carrots and apricots*.—Grind material in a food chopper. Weigh 5 grams and reflux with alcoholic KOH as directed in 61, p. 369, *Methods of Analysis*, A. O. A. C., 1940, except to triturate the residue with petroleum benzine and alcohol until no more color is removed.

(d) *Fresh sweet potatoes*.—Proceed as directed in 61, p. 369, *Ibid.*, except to reflux with 95% ethyl alcohol instead of alcoholic KOH.

(e) *Dehydrated fruits and vegetables containing high amount of sugar (carrots, sweet potatoes, apricots, etc.)*.—To a 2–5 gram sample, add 20–50 ml. of distilled water and allow to stand overnight in refrigerator. Then proceed as directed in 61, p. 369, *Ibid.* (Use of a Waring blender or similar type of apparatus is helpful in the extraction of these samples.)

(f) *High lycopene-containing materials (tomatoes and water melons)*.

\* For report of Subcommittee A and action by the Association, see *This Journal*, 27, 48 (1944).

## REAGENTS

*Magnesium carbonate*.—Use U.S.P. light  $\text{MgCO}_3$  that will adsorb less than 5% of carotene when tested by the following method:

Place approximately 1 gram in a tube as directed below and pass through it a solution of purified carotene (*This Journal*, 24, 860), containing 1.0–1.5 p.p.m. of carotene. Wash with the petroleum benzine and determine the carotene in the filtrate. If the  $\text{MgCO}_3$  is too retentive of carotene, try another lot.

## PREPARATION OF COLUMN

Place 1 gram of the  $\text{MgCO}_3$  in a glass tube 5–8 mm. wide and about 15 cm. tall, constricted at one end and plugged with a wad of cotton. Apply suction and pack the  $\text{MgCO}_3$  firmly but not too tightly with a cork having a smooth surface and attached to a glass rod.

## DETERMINATION

Weigh 5 grams of sample and obtain the crude carotene as directed in 61, 369, *Methods of Analysis*, A. O. A. C., 1940. Dilute to 200 ml. Evaporate 50 ml. aliquot in vacuo to approximately 10 ml. Then run through a column of  $\text{MgCO}_3$ . Wash the column with petroleum benzine until all the carotene is washed through. (A red band of lycopene will pass slowly down the column. Be careful that none of this pigment is washed through the column.) Dilute the solution that passes through the column to appropriate volume and determine the amount of crude carotene as directed in 61, 369, *Ibid*.

(g) *Canned foods*.—Pour contents of the can onto a piece of cheese cloth and allow liquid to drain. Grind the solids in a food chopper and proceed as directed in 61, p. 369, *Ibid*.

(h) *Butter and other fats, egg yolks and liver*.—Weigh 5 gram sample and proceed as directed in 61, 369, *Ibid*.

(i) *Blood plasma*.—Weigh 10–30 grams of blood plasma and reflux with 25–50 ml. of alcoholic KOH for 15 minutes. Proceed as directed in 61, 369, *Ibid*.

(2) That the complete chromatographic method described below be tentatively adopted.

## CAROTENE

*Chromatographic Method*

## 1

## REAGENTS

(a) *Alcoholic potash*.—Dissolve 12 grams of KOH in 100 ml. of 95% ethyl alcohol.

(b) *Petroleum benzine*.—Use petroleum benzine, b.p. 30°–70°C., or Skellysolve F, b.p. 86°–170°F. (30°–70°C.).

(c) *Methanol*.—90%. Dilute 100 ml. of water in a liter flask to volume with absolute methanol.

(d) *Calcium hydroxide*.—Use ordinary commercial hydrated lime that has been sifted through a 48–65-mesh sieve.

## 2

## PREPARATION OF ADSORPTION COLUMN

Prepare the adsorption column as directed in *This Journal*, 25, 886, except to use the  $\text{Ca(OH)}_2$  instead of  $\text{MgO}$  and omit cooling the column with ice water.

## 3

## EXTRACTION OF PIGMENTS

Avoid the use of methods in which heating is used because heating produces neo-beta-carotene.<sup>3</sup>

(a) *Fresh materials, butter, egg yolk, and fresh or dried fecal material.*—Chop the fresh material into pieces about  $\frac{1}{2}$ " long and weigh 10 grams into the chamber of a Waring blender or similar type of apparatus. Add 150–200 ml. of the alcoholic KOH and grind for 5 minutes. Extract with petroleum benzine and wash with methyl alcohol as directed in 61, 369, *Methods of Analysis*, A. O. A. C., 1940.

(b) *Dried hays, grass, and dehydrated leafy vegetables.*—To 5–25 grams of material, depending on carotene content, add 150–200 ml. of alcoholic KOH, and let stand overnight in refrigerator. Then grind in a Waring blender or similar type of apparatus and extract as directed in 3(a).

(c) *Fresh sweet potatoes.*—Proceed as directed for other fresh materials, 3(a), except to use 95% ethyl alcohol in place of the alcoholic KOH.

(d) *Dehydrated fruits or vegetables high in sugar, such as dried apricots, carrots, or sweet potatoes.*—Weigh a 5–10 gram sample, add 50–100 ml. of water, and allow to stand overnight in refrigerator. Then proceed as directed in 3(a).

## 4

## SEPARATION OF PIGMENTS

Separate the chromatographic pigments as directed in the method for yellow corn published in *This Journal*, 25, 887. With most materials, the bands of pigments found in the columns starting from the top are as follows:

(a) *Impurity A.*—May consist of several bands of yellow, red, or brownish yellow pigments.

(b) *Carotenoid X.*—A light orange pigment just above the beta carotene band. This pigment separates late in the analysis.

(c) *Beta carotene.*—A wide reddish orange band.

(d) *Neobeta carotene.*—A yellow-orange band immediately below the beta carotene band.

(e) *Alpha carotene.*—An orange band that is not present in most materials in appreciable amounts.

Another small band is sometimes present below the alpha carotene band. For all practical purposes it may be removed with the alpha carotene. Sometimes carotenoid X separates from the other pigments very slowly. Washing the column with petroleum benzine containing 1–5% acetone is often helpful.

## REPORT ON INSECTICIDES AND FUNGICIDES

By J. J. T. GRAHAM (Insecticide Division, Livestock and Meats Branch, Food Distribution Administration, Beltsville, Md.), *Referee*

The mercury reduction method for determination of Pyrethrin I in pyrethrum powder and extracts proposed by Wilcoxon<sup>1</sup> and modified by Holaday<sup>2</sup> has been adopted by this Association as an official method.<sup>3</sup>

<sup>1</sup> *Contrib. Boyce Thompson Inst.*, 8, 175 (1936).

<sup>2</sup> *Ind. Eng. Chem., Anal. Ed.*, 10, 5 (1938).

<sup>3</sup> *Methods of Analysis*, A.O.A.C., 1940, 66.

In the commercial use of this method it appeared that the results obtained were too low. A reinvestigation of the relation between the reduced mercury and Pyrethrin I has been made by Graham and LaForge.<sup>4</sup>

For the investigation upon which the method was based, Wilcoxon<sup>1</sup> prepared a sample of chrysanthemum monocarboxylic acid which was 99.8 per cent pure. The purity and identity of this sample were established by alkali titration, microchemical combustion, and analysis of the silver salt of the acid. Using this preparation Wilcoxon found that under the conditions of his experiments, three atoms of Mercury (I) was equivalent to 1 mol of the chrysanthemum acid, and therefore of Pyrethrin I. According to Jamieson<sup>5</sup> 1 mol of iodate is equivalent to four atoms of Mercury (I). From these equivalents, Wilcoxon derived the factor 1 ml. of 0.01M  $KIO_3 = 4.4$  mg. of Pyrethrin I, and this factor has been used in the official method to the present time.

In their investigation of the relation between Pyrethrin I and the reduced mercury, Graham and LaForge used four samples of pure acid, three of which were prepared as described by LaForge and Haller<sup>6</sup> and one that was prepared as described by Wilcoxon.<sup>1</sup> The purity and identity of these samples were established by titration, refractive index, catalytic hydrogenation, and combustion (C and H) analysis.

Using the four samples of pure acid, these investigators report nineteen closely agreeing results from which they find that 1 ml. of 0.01M  $KIO_3 = 5.70 (\pm .02)$  mg. of Pyrethrin I, and they recommend the adoption of the factor 5.70 for use with this method.

In consideration of this work it appeared necessary to have cooperative work done on it in order that steps might be taken by the Association to adopt this modification. Samples of the pure acid were therefore sent to several collaborators with the following directions for analysis:

Weigh approximately 0.09 gram of the pure chrysanthemum monocarboxylic acid in a weighing capsule. Transfer the capsule and contents to a 50 ml. beaker and add slowly with constant stirring 0.1 N (approx.) NaOH in alcohol colored with a drop or two of phenolphthalein solution. Add the NaOH solution in slight excess. Transfer the solution of the sodium salt of chrysanthemum monocarboxylic acid to a 50 ml. volumetric flask and make to volume. Pipet 10 ml. aliquots of this solution into 100 ml. beakers, add 10 ml. of Deniges reagent, and allow to stand 1 hour. Add 20 ml. of alcohol and 3 ml. of saturated NaCl solution and heat on steam bath for a few minutes (until the precipitate has flocculated). Filter through a 7 cm. paper (S&S No. 589 or like grade of other filters), and wash twice with hot alcohol and three times with hot  $CHCl_3$ . Transfer filter and contents to a glass-stoppered flask and titrate with 0.01 M  $KIO_3$  solution as directed in the official method (*Methods of Analysis*, A. O. A. C., 1940, 67).

*Remarks.*—In reporting the results, give weight of the pure acid in each aliquot

<sup>4</sup> *Soap*, 19, 111 (1943).

<sup>5</sup> "Volumetric Iodate Methods," Chemical Catalog Co., New York (1926).

<sup>6</sup> *J. Org. Chem.*, 2, 59 (1937).



and the number of ml. of 0.01 *M* KIO<sub>3</sub> required for each titration. Report individual determinations, not averages. Correct the titrations if necessary by subtracting a blank. It has been the experience of the Referee that the Mercury (I) in the Deniges reagent usually requires a correction of about 0.1 ml. in the quantity of KIO<sub>3</sub> solution used for titration.

The results submitted by the collaborators are given in Table 1.

TABLE 1.—*Collaborative results on chrysanthemum monocarboxylic acid, calculated to Pyrethrin I*

ANALYST	QUANTITY TAKEN		0.01 <i>M</i> KIO <sub>3</sub>	DERIVED FACTOR, PYRETHRIN I
	CHRS. ACID	EQUIV. PYR. I		
	mg.	mg.	ml.	mg./ml.
J. J. T. Graham	19.71	38.71	6.80	5.69
	19.71	38.71	6.78	5.71
				Av. 5.70
W. F. Barthel	19.71	38.71	6.80	5.69
Bur. Ent. & Plant	19.71	38.71	6.82	5.68
Quarantine	18.10	35.55	6.30	5.64
Beltsville, Md.	18.10	35.55	6.18	5.75
				Av. 5.69
S. B. Soloway	21.48	42.19	7.30	5.78
Bur. Ent. & Plant	37.56	73.78	12.60	5.86
Quarantine	37.56	73.78	12.54	5.88
Beltsville, Md.	—	—	—	—
				Av. 5.84
Bur. of Chem.	18.64	36.61	5.98	6.12
State of Calif.	18.58	36.50	6.02	6.06
Dept. of Agr.	36.58	71.85	11.88	6.05
Sacramento, Calif.	18.64	36.61	6.12	5.98
	18.58	36.50	6.15	5.93
				Av. 6.03

An examination of the results in Table 1 shows that they are in substantial agreement with those reported by Graham and LaForge.<sup>4</sup>

#### DISCUSSION

As previously stated, Wilcoxon<sup>1</sup> found that 1 mol of chrysanthemum monocarboxylic acid reduced 3 atoms of mercury. Graham and LaForge pointed out that it is unlikely that the breaking of a double bond would

result in the reduction of an odd number of atoms of mercury and stated that it was reasonable to assume that Wilcoxon's factor was an empirical one. They found that in their work the number of atoms of mercury reduced was 2.38, which was not a whole number, but that the reaction did reach a definite and reproducible end point. They suggested that the empirical factor 1 ml. of 0.01  $M$   $KIO_3$  = 5.70 mg. of Pyrethrin I be used in the method. The work of the collaborators given in this report supports their suggestion.

#### RECOMMENDATIONS\*

It is recommended that the value 1 ml. of 0.01  $M$   $KIO_3$  = 5.70 mg. of Pyrethrin I be adopted as the factor in the mercury reduction method<sup>a</sup> for Pyrethrin I in pyrethrum powder and extracts (first action).

### REPORT ON FLUORINE COMPOUNDS

By C. G. DONOVAN (Insecticide Division, Livestock and Meats Branch, Food Distribution Administration, Beltsville, Md.), *Associate Referee*

At the annual meeting in 1939, this Association adopted the lead chlorofluoride procedure as an official method for the determination of fluorine in insecticides. This method has proved to be more satisfactory and can be applied to a larger variety of fluorine samples than any other method yet developed. However, the method, as originally adopted contains several unnecessary steps that prolong the time of analysis, and it lacks sufficient directions for its determination in certain other types of insecticides now being marketed. Since the formulas of the fluorine insecticides are so varied, it appears impractical to have one general lead chlorofluoride procedure applicable to the various mixtures. The report this year consists chiefly in offering the following more detailed method, which is divided into five procedures for the different types of fluorine insecticides and which is based on the collaborative and investigational analyses conducted by the Associate Referee with lead chlorofluoride procedures.

#### FLUORINE

##### *Lead Chlorofluoride Method*

#### REAGENTS

(a) *Fusion mixture*.—Mix anhydrous  $Na_2CO_3$  and  $K_2CO_3$  in equimolecular proportions.

(b) *Lead chlorofluoride wash solution*.—Dissolve 10 grams of  $Pb(NO_3)_2$  in 200 ml. of water; dissolve 1 gram of  $NaF$  in 100 ml. of water and add 2 ml. of  $HCl$ ; mix these 2 solutions. Allow precipitate to settle and decant supernatant liquid. Wash 4 or 5 times with 200 ml. of water by decantation, and then add about 1 liter of cold water

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 27, 48 (1944).

to the precipitate and allow to stand 1 hour or longer, with occasional stirring. Pour through filter and use clear filtrate. By adding more water to the precipitate of  $\text{PbClF}$  and stirring, more wash solution may be prepared as needed.

(c) *Standard silver nitrate solution*.—0.1 or 0.2 N. Standardize by titration against pure  $\text{NaCl}$ , using  $\text{K}_2\text{CrO}_4$  indicator.

(d) *Standard potassium or ammonium thiocyanate solution*.—0.1 N. Standardize by comparing with the standard solution of  $\text{AgNO}_3$  under the same conditions as obtain in the determination.

(e) *Ferric indicator*.—Add to cold saturated solution of ferric alum (free from  $\text{Cl}$ ) sufficient colorless  $\text{HNO}_3$  to bleach the brown color.

(f) *Bromophenol blue indicator*.—Grind 0.1 gram of the powder with 1.5 ml. of 0.1 N  $\text{NaOH}$  solution and dilute to 25 ml.

#### DETERMINATION

I. *Samples difficult to decompose such as cryolite, and others that contain aluminum or appreciable quantities of silicious material*.—Mix 0.5 gram (or less if necessary to make content of  $\text{F}$  fall between 0.01 and 0.10 gram) of sample with 5 grams of fusion mixture and 0.2–0.3 gram of powdered silica, cover with 1 gram of fusion mixture, and heat to fusion over Bunsen burner. (Use of blast lamp is not required as it is only necessary that the mass be fluid, and it is preferable not to heat much beyond the temperature at which it melts. If much  $\text{Al}$  is present, a uniform, clear, liquid melt cannot be obtained. There will be particles of a white solid separated in the liquid. The melt after cooling should be colorless, or at least should not have more than a gray color.)

Leach cooled melt with hot water and filter into a 400 ml. beaker when disintegration is complete. Return the insoluble residue to a Pt dish by the use of jet of water, add 1 gram of  $\text{Na}_2\text{CO}_3$ , make to volume 30–50 ml., boil a few minutes, disintegrating any lumps with glass rod flattened on end, filter through same paper, wash thoroughly with hot water, and adjust volume of filtrate and washings to approximately 200 ml. Add 1 gram of  $\text{ZnO}$  dissolved in 20 ml. of  $\text{HNO}_3$  (1+9), boil 2 minutes with constant stirring, filter, and wash thoroughly with hot water. During this washing return the gelatinous mass to the beaker three times and thoroughly disintegrate in the wash solution because it is difficult to wash this precipitate on filter. (The mass can easily be returned to beaker by rotating funnel above beaker and at the same time cutting precipitate loose from paper with jet of wash solution.)

Add 2 drops of bromophenol blue and, with a cover-glass almost over the beaker, add  $\text{HNO}_3$  (1+4) until the color just changes to yellow. Make solution slightly alkaline with 10%  $\text{NaOH}$  and boil gently, with cover-glasses on the beakers, to expel  $\text{CO}_2$ . Remove from burner; add  $\text{HNO}_3$  (1+4) until color just changes to yellow and then dilute  $\text{NaOH}$  until color just changes to blue; and add 3 ml. of 10%  $\text{NaCl}$ . (Volume of solution at this point should be 250 ml.)

Add 2 ml. of  $\text{HCl}$  (1+1) and 5 grams of  $\text{Pb}(\text{NO}_3)_2$  and heat on steam bath. As soon as the  $\text{Pb}(\text{NO}_3)_2$  is in solution add 5 grams of sodium acetate, stir vigorously, and digest on steam bath 30 minutes with occasional stirring. Allow to stand overnight at room temperature. Decant solution through a paper of close texture; wash precipitate, beaker, and paper once with cold water, then 4 or 5 times with a cool saturated solution of  $\text{PbClF}$ , and then once more with cold water.

Transfer precipitate and paper to beaker in which precipitation was made, stir paper to a pulp, add 100 ml. of  $\text{HNO}_3$  (5+95), and heat on steam bath until precipitate is dissolved (5 minutes is ample to dissolve this precipitate. If sample contains an appreciable quantity of sulfates the precipitate will contain  $\text{PbSO}_4$ , which will

not dissolve. In such a case heat 5-10 minutes with stirring and consider the  $\text{PbClF}$  to be dissolved). Add a slight excess of 0.1 *N* or 0.2 *N*  $\text{AgNO}_3$  solution, digest on steam bath 30 minutes, cool to room temperature while protected from light, filter, wash with cold water, and determine  $\text{AgNO}_3$  in the filtrate by titration with the standard thiocyanate solution, using 10 ml. of the ferric indicator. Subtract quantity of  $\text{AgNO}_3$  found in filtrate from that originally added. The difference will be that required to combine with the Cl in the  $\text{PbClF}$ , and from this difference calculate percentage of F in sample on basis that 1 ml. of 0.1 *N*  $\text{AgNO}_3$  = 0.0019 gram of F, or 0.2 *N*  $\text{AgNO}_3$  = 0.00380 gram of F.

II. *Water-soluble fluorides in presence of up to 50% organic matter such as flour, pyrethrum, tobacco powder, and derris or cube powders, which are readily decomposed without addition of powdered silica and are free from or contain only small quantities of aluminum or silicious compounds.*—Mix 0.5 gram (or less if necessary to make content of F fall between 0.01 and 0.1 gram) of sample with 5 grams of fusion mixture, cover with 1 gram of fusion mixture, and heat to fusion over Bunsen burner. Leach cooled melt with hot water, filter into a 600 ml. beaker when disintegration is complete, and wash thoroughly with hot water. Proceed as directed under I beginning, "Add 2 drops of bromophenol blue and then with cover-glass almost over the beaker add dilute  $\text{HNO}_3$  (1+4) until the color just changes to yellow."

III. *Water-soluble samples in absence of organic matter and appreciable quantities of sulfates or aluminum salts.*—The fusion may be omitted in absence of organic matter or other interfering substances and determination made on an aliquot of a water-soluble solution containing between 0.01 and 0.1 gram of F, as directed in I, beginning "Add 2 drops of bromophenol blue."

In presence of aluminum, as in sample containing sodium silicofluoride and potassium aluminum sulfate, transfer sample to 400 ml. beaker, dissolve in 150 ml. of hot water, add 6 grams of fusion mixture, and boil. Add 1 gram of  $\text{ZnO}$  dissolved in 20 ml. of  $\text{HNO}_3$  (1+9), boil 2 minutes with constant stirring, filter into a 500 ml. volumetric flask, and wash thoroughly with hot water. Cool to room temperature and make to volume. Transfer a 200 ml. aliquot containing 0.01-0.10 gram of F to a 600 ml. beaker, and proceed as directed in I, beginning "Add 2 drops of bromophenol indicator."

IV. *Sodium and magnesium silicofluorides in absence of aluminum and boron, with or without organic matter.*—With large quantities of sodium silicofluoride and some of the more volatile silicofluorides, for example Mg, where there is a possibility of some of the F being evolved as  $\text{SiF}_4$  before the melt with the fusion is effected, distil the F as directed in the A.O.A.C. method (*Methods of Analysis, A.O.A.C.*, 1940, p. 51) and determine the F in the distillate. Add several drops of bromophenol blue, make alkaline with  $\text{NaOH}$ , and adjust volume to about 250 ml. by gently boiling the volume down from 400 to 250 ml. Proceed as directed under I, beginning "Remove from burner, add  $\text{HNO}_3$  (1+4)."

V. *Samples containing large quantities of organic matter or appreciable quantities of sulfates.*—Proceed as directed in IV. If sample contains over .8 gram of organic matter, make distillate alkaline with  $\text{NaOH}$ , evaporate to 25-30 ml., and redistil.

NOTES: These procedures give accurate results for 0.01-0.10 gram of F. Below 0.01 gram the results have a tendency to be slightly low and above 0.10 slightly high. A convenient sample to fuse is one that contains 0.07-0.08 gram of fluorine; however, it is inadvisable to use too large a sample as an incomplete fusion may result. Large quantities of boron compounds and alkali salts retard or prevent the complete precipitation of lead chlorofluoride. Boron has a greater effect when the quantity of F is large than when it is small. In the described procedures the effect of boron is small and may be disregarded in the analysis of insecticides if the amount of F to be precipitated is not above 0.03 gram. With some compounds containing borax or boric acid, where it is difficult to obtain a representative mixture when an extremely

small sample (0.1 gram) is used for analysis, a larger one may be taken and the lead chlorofluoride precipitated from an aliquot of the fusion solution. The quantity of alkali carbonates specified in the fusion and in the washing of the insoluble residue is not large enough to cause low results.

If sample contains S, it should be removed with CS<sub>2</sub> and F determined on air dry residue, allowance being made in calculations for percentage of S removal.

### ANALYSIS OF TEST SAMPLES

A commercial cryolite and four especially prepared samples were used for collaborative analysis. The composition, the theoretical value for the total fluorine in the prepared samples, and the results of the total fluorine determined by three of the procedures described are shown in Table 1.

TABLE 1.—Results on prepared samples

COMPOSITION (PARTS/100)	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5
	COMMER- CIAL CRYOLITE	BARIUM FLUO- SILICATE 20 FULLERS' EARTH 80	SODIUM FLUORIDE 60 PYRETHRUM POWDER 25 CELITE 10 FLOUR 5	SODIUM FLUORIDE 45 SODIUM SILICO- FLUORIDE 5 PYRETHRUM POWDER 50	SPECIALLY PREPARED SODIUM FLUORIDE
Theoretical values (Per cent F)		7.78	26.77	23.09	45.06
Procedure used	I	I	II	II	III
Analyst R. L. Caswell San Francisco Av.	50.79 50.60 50.70		27.02 26.94 26.98	23.26 22.78 23.02	
C. G. Donovan Beltsville, Md. Av.	50.20 50.20 50.20	7.76 7.76	26.53 26.30 26.42	22.78 22.64 22.71	45.11 45.27 45.01 45.13 45.13
J. J. T. Graham Beltsville, Md. Av.		7.86 7.78 7.70 7.78			44.98 45.10 45.04
E. C. Payne Chicago Av.	50.2 51.1 50.65		27.13  27.13	23.15  23.15	
State of California, Dept. of Agr. Av.	50.50 50.75 50.63		26.52 26.52 26.52	22.90 23.02 22.96	
Analyst A State of Maryland, Inspection and Regulatory Service Av.	50.45 50.43 50.44	7.78	27.20	23.25  23.25	
Analyst B State of Maryland, Inspection Service Av.	50.69 50.75 50.72		27.14	23.24  23.24	
General Av.	50.56	7.78	26.81	23.00	45.10

Other mixtures of known composition analogous in composition to proprietary preparations on the market were prepared, and the fluorine then determined by the Associate Referee by the five procedures described. The results of these determinations, given in Table 2, are typical of those obtained in commercial mixtures.

TABLE 2.—Results on fluorine insecticides

COMPOSITION (PARTS/100)	PROCEDURE	SAMPLE	FLUORINE (%)	
			PRESENT	FOUND
Sodium fluoride 50 Pyrethrum 25 Celite 25	I	gram		
		.10	22.34	22.23
Sodium fluoride 50 Wheat flour 50	II	.30	22.34	22.36
Sodium fluoride 60 Borax 20	II	.10	26.81	26.64
Pyrethrum powder 10 Celite 10	II	.30	26.81	*26.84
Sodium silicofluoride 66½ Potassium Aluminum sulfate 33½	III	.36	40.19	40.18
Sodium silicofluoride 50 Wheat flour 50	IV	.25	30.14	30.07
Sodium fluoride 15 Pyrethrum powder 85	V	1.00	6.70	6.84
		0.50	6.70	6.82

\* Lead chlorofluoride precipitated from an aliquot of the fusion solution.

## DISCUSSION

Procedure I.—In the presence of excessive quantities of silica some silica precipitates with the lead chlorofluoride and retards or entirely prevents its filtration. The silica may also carry down chlorine and this causes high results. Low results are obtained in the presence of aluminum. It is necessary therefore with samples of this nature to remove most of the silica and aluminum from the water solutions after fusion. This procedure is essentially the official lead chlorofluoride method. The results of the collaborative investigation were given in the 1939 report on fluorine compounds, which was published in *This Journal*, 23, 547 (1940).

Procedure II.—In the analysis of the mixtures for which Procedure II should be used it is unnecessary to add silica to complete the fusion, or to

add the zinc oxide-nitric acid solution, which eliminates two troublesome steps, namely the filtering and washing of the bulky precipitate formed by the addition of the zinc solution and the subsequent adjustment of the filtrate to proper volume by boiling.

Procedure III.—The first part, for samples of the nature of commercial sodium fluoride, was incorporated in the official method that was published in *Methods of Analysis*, A.O.A.C., 1940, as a note. The second part pertains to the analysis of certain insecticides that contain sodium silicofluoride and potassium aluminum sulfate. By this procedure, the fusion which causes low results with large quantities of sodium silicofluoride is avoided, and the complete removal of aluminum and silicon is attained by precipitation from an aqueous solution with sodium carbonate and zinc oxide-nitric acid solution.

Procedure IV—It is not advisable to fuse samples of this nature as some of the fluorine is evolved as silicon tetrafluoride before the melt is complete. In this procedure the fluorine is distilled as fluosilicic acid and precipitated as lead chlorofluoride from the distillate.

Procedure V.—As examples for the application of this procedure may be cited mixtures of 85 per cent pyrethrum powder and 15 per cent sodium fluoride, or mixtures of commercial sodium fluoride with 5–10 per cent sodium sulfate. It is inadvisable to use Procedures I or II with samples containing over 50 per cent organic matter since the time required for the satisfactory ignition of the organic matter is too long and some fluorine is lost in the prolonged heating. When the determination is made on a sample containing over .8 gram of organic matter of the nature of pyrethrum powder or starchy material such as corn meal or wheat flour excessive quantities of fatty material distil over, which causes high results. By this procedure the fluorine is separated from the sulfate before the precipitation.

It is recommended\* that in the lead chlorofluoride method for determination of total fluorine, Procedures I, II, III, IV, and V be adopted as a substitute for the lead chlorofluoride method, *Methods of Analysis* A.O.A.C., 1940, which is now official for this determination.

## REPORT ON DISINFECTANTS

By C. M. BREWER (Insecticide Division, Livestock and Meats Branch, Food Distribution Administration, Beltsville, Md.), *Referee*

In view of the criticism lately aimed at the phenol coefficient method and the dissatisfaction now being expressed with phenol coefficient results, it seems pertinent at this time to reconsider briefly the official procedure, *Methods of Analysis*, A.O.A.C., 1940, 75, and its purpose.

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 27, 48 (1944).

There is little objection to the method as a test for coal tar, cresylic, and pine oil types of products, which until quite recently formed the preponderance of disinfectants found on the market. The main objections result from the use of the method for testing synthetic preparations now being offered in rapidly increasing amounts as substitutes for carbolic acid and cresylic compounds.

As a test for some of these synthetic products, the official phenol coefficient method has been found to give irregular results, and these have emphasized certain weaknesses in the method. Thus far three features of the test have been recognized as mainly responsible for its inaccuracies. (a) The media: The regulation peptone has not proved consistent in composition. Small changes in the phospholipid content of different batch lots used in culture medium have been shown to exert a profound effect on the phenolic coefficient results obtained on certain synthetic detergents and coal tar disinfectants fortified with synthetic wetting agents.<sup>1</sup> Also the pH of the test cultures as affected by components of the peptone may also strongly influence the phenol coefficient values of certain synthetic compounds, especially the halogenated phenols.<sup>2</sup> (b) The test organism: The tendency of the test organism to mutate between rough and smooth form has been reported to affect the phenol coefficient values of certain synthetic preparations.<sup>3</sup> (c) The transfer loop: Owing to different surface tension values exhibited by the variety of current products used as disinfectants, the use of the standard transfer loop results in the removal of different amounts of test material with a consequent difference in the number of treated organisms carried into the sub-cultures, and constitutes an appreciable source of error.

To point out that the limited applicability of the official method has long been recognized, and that it continues to be satisfactory for many types of disinfectants, is only to sidestep the responsibility of improving the procedure to accommodate the testing of widely different materials for which there is now no suitable test. It is expected that correction of the features just cited will do much to broaden the utility of the method and improve its accuracy. Work on these problems is now under way, and they are the subject of an intensive collaborative effort. In support of the test it might be said that much of the criticism of the method that has not been justified has emanated from the misconception that the phenol coefficient method should provide a means of determining the special characteristics and the selective action of a disinfectant—in short its practical value. The multiplicity of conditions and places in which germicides may be subjected to use precludes any possibility of obtaining such information from a single laboratory test. A chemical method has the single

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<sup>1</sup> Brewer, C. M., *Am. J. Public Health*, 33, 261-264 (1943).

<sup>2</sup> Unpublished data.

<sup>3</sup> Mallmann, W. L., Mich. State Coll., E. Lansing, Mich. Unpublished.



purpose of determining the presence or amount of a specific compound; the assay of a drug determines the degree of activity of a specific biological principle; just so, the scope of the phenol coefficient method should be limited to the measurement of the germicidal potentialities possessed by a material. The utility and means of best exploiting the germicidal potentiality can be made known only through a number of supporting tests; whether it is advisable to grant official recognition to certain accessory bacteriological methods of testing disinfectants is beside the point. However, disastrous confusion in the field of germicidal testing can be avoided only by maintaining a means of determining the value of the germicidal power of a compound under strictly standardized conditions, to serve as a reference point for comparative purposes and a basis for related experimentation.

With the advent of numberless formulations possessing bactericidal activity and with the U. S. Department of Agriculture Circular 198, describing the F.D.A. phenol coefficient method, now out of print, the official status of the present phenol coefficient method seems particularly important at this time.

## REPORT ON STANDARD SOLUTIONS

By R. L. VANDAVEER (Food and Drug Administration,  
Federal Security Agency, New Orleans, La.), *Referee*

No reports were received this year on methods of standardization of thiocyanate solution; therefore, it is recommended that both of the subjects stated below be studied further next year, although in the first-named subject a report is submitted by the Associate Referee. In connection with this report the Associate Referee recommends that the preparation and standardization of sodium thiosulfate solutions by potassium dichromate be submitted for collaborative study. The Referee concurs in this recommendation.

(1) Method for preparation and standardization of sodium thiosulfate solution.

(2) Methods of standardization of thiocyanate solution.

Consistent with the Referee's recommendation last year, it is again recommended\* that the methods for the preparation of standard hydrochloric acid solution from constant boiling acid and sodium hydroxide solutions by means of constant boiling hydrochloric acid be adopted as official, final action.

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 27, 48 (1944).

## REPORT ON STABILITY OF STANDARD SODIUM THIOSULFATE SOLUTIONS

By GEORGE M. JOHNSON (Food and Drug Administration,  
Federal Security Agency, St. Louis, Mo.), *Associate Referee*

The sterilization of sodium thiosulfate solutions by boiling gives rise to the question whether decomposition occurs. According to Kolthoff,<sup>1</sup> the reactions involved in the decomposition of thiosulfates are  $\text{Na}_2\text{S}_2\text{O}_3 \rightarrow \text{Na}_2\text{SO}_3 + \text{S}$  (very slow) and  $\text{Na}_2\text{SO}_3 + \text{O} \rightarrow \text{Na}_2\text{SO}_4$  (measurable). It was thought that boiling might accelerate the formation of sulfite.

In an effort to determine the effect of boiling on thiosulfate solution, two liters of approximately 0.1 *N* sodium thiosulfate was prepared with boiled and cooled distilled water. One liter of this solution was left as it was; two other portions were boiled under a reflux condenser, for 5 and 15 minutes, respectively, and transferred while hot to Pyrex bottles previously cleaned with  $\text{Na}_2\text{Cr}_2\text{O}_7 + \text{H}_2\text{SO}_4$  solution and dried in an oven at 100°C.

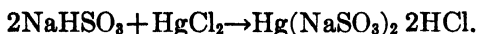
Each of these solutions was standardized by the proposed A.O.A.C. method, and the sulfite content was determined by the method of Feld<sup>2</sup> and Sander.<sup>3</sup> This method depends on the liberation of acid when mercuric chloride is added to a solution of thiosulfate.



Sodium sulfite reacts similarly, forming a complex mercury salt, but does not give free acid.



Sodium bisulfite reacts with mercuric chloride to give a complex sulfonic acid which has strong acid properties.



In the solution under consideration, however, it was found by direct acid and alkali titration<sup>4</sup> that only negligible amounts of bisulfites were present, and therefore all the liberated acid could be calculated to thiosulfate. The results obtained on these solutions are summarized as follows:

	NO. 1	NO. 2	NO. 3
Solution	Not Boiled	5 min. Boiling	15 min. Boiling
Normality ( $\text{K}_2\text{Cr}_2\text{O}_7$ )	0.1012	0.1015	0.1016
$\text{Na}_2\text{SO}_3$ (mg/100 ml.)	4.0	5.0	6.0
Free sulfur	none	none	none

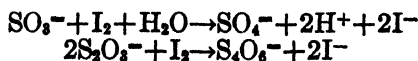
<sup>1</sup> "Volumetric Analysis," Vol. 1.

<sup>2</sup> *Z. Angew. Chem.*, 29, 1161 (1911); *Analyst*, 36, 435 (1911).

<sup>3</sup> *Chem. Z.*, 39, 945 (1915); *Analyst*, 41, 84 (1916); *Z. anal. Chem.*, 55, 340 (1916); *Analyst*, 41, 320 (1916).

<sup>4</sup> Bonshard and Grab, *Chem. Z.*, 37, 465 (1913); *Analyst*, 38, 297 (1913).

It will be noted that changes in the solutions on boiling are slight. Sulfites increase the normality of a solution as will be noted by the equations.



It is believed, however, that the slight increase in normality of the boiled solutions is due to the unavoidable loss of vapor when the hot solutions were transferred to the Pyrex bottles. The small variation in sulfite content does not appear significant. In support of these conclusions it may be pointed out that W. H. McCown<sup>5</sup> finds that autoclaving 0.1 *N* solutions for five and fifteen minute periods under fifteen pounds' pressure caused no change in titer.

The Associate Referee has prepared a number of 0.1 *N* solutions by the proposed A.O.A.C. method over a period of several years. He has found that change in titer under conditions of normal use does not exceed one or two parts per million in six months.

The primary purpose of boiling thiosulfate solutions is to prevent bacterial decomposition without resorting to chemical preservatives. This, of course, insures sterility of the solution when prepared and requires careful handling of the solution during use to minimize danger of inoculation. Unused portions of the solution should be discarded and should not be returned to the stock bottle. The stopper of the bottle should be held in the fingers and not be allowed to touch the table top. Pipets or other pieces of apparatus should not be introduced into the stock bottle.

Chemical decomposition is not prevented by boiling of the solution. It is evidenced in a solution by the presence of free sulfur, a decrease in pH, the formation of sulfates, and in some cases the formation of sulfides.<sup>6</sup> The chemical change is slow, however; solutions stored for 22 months showed a decrease in normality of about one per cent.

The distilled water should be of good quality. Kilpatrick and Patrick<sup>7</sup> find that redistilled water gives greater stability than laboratory distilled water. This may be due to removal of copper, which in minute traces catalyzes the decomposition of thiosulfates. Therefore, water stored in copper containers should not be used if maximum stability is desired. The optimum conditions for maximum stability seem to be as follows: Water, salt, and container should be sterile; the distilled water should be of good quality; and the stock solution should be stored in a dark, cool place.

It is recommended that the preparation and the standardization of sodium thiosulfate solutions by potassium dichromate be submitted to collaborative study.

<sup>5</sup> Private communication from W. H. McCown, Food and Drug Adm., New Orleans, La.

<sup>6</sup> Rue, S. D., *Ind. Eng. Chem., Anal. Ed.*, 14, 802 (1942).

<sup>7</sup> *J. Am. Chem. Soc.*, 45, 2132 (1923).

## CONTRIBUTED PAPERS

### DILUTE HYDROCHLORIC ACID AS A SOLVENT FOR PHOSPHATES WITH SPECIAL REFERENCE TO DEFLUORINATED PHOSPHATES AND OTHER MATERIALS USED AS PHOSPHORUS SUPPLEMENTS FOR LIVESTOCK

By D. S. REYNOLDS, W. L. HILL and K. D. JACOB (Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, Beltsville, Maryland)

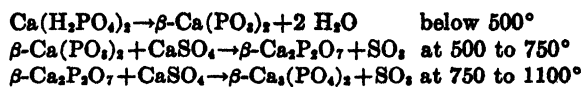
The shortage of bone products for use as phosphorus supplements in animal feeds has focused attention on the production of substitute materials, among which thermally defluorinated superphosphate and thermally defluorinated phosphate rock are of special interest (2, 7, 8, 10, 12, 13, 14, 20). With the new materials comes the question of phosphorus assimilability in comparison with bone meal, but feed control officials, being accustomed to a plentiful supply of bone meal, have not found it necessary to devise a chemical method for determining assimilable phosphorus in mineral feeds. Consequently, there is no standard procedure by means of which these substitute materials may be quickly compared with one another or with materials of known value. Nevertheless, extraction with dilute hydrochloric acid within the range of concentrations found in the stomachs of animals has been used by workers in this field, although a standardized procedure apparently has not been evolved. The work reported in this paper was initiated to study the factors involved in the extraction of phosphates with dilute hydrochloric acid, with special reference to thermally defluorinated phosphates.

#### NATURE OF DEFLUORINATED PHOSPHATES

*Defluorinated superphosphate.*—The ordinary superphosphate manufactured in this country at the present time is essentially a mixture of anhydrous calcium sulfate (anhydrite) and monocalcium phosphate (9) that usually carries 1.2–2.0 per cent of fluorine, which, on account of its toxicity to animals (15, 18), must be removed before the material can be safely used as a mineral feed. In order to remove the fluorine, the superphosphate is heated in a rotary kiln at 600°–800°C. (21, 23) or higher (22), and in the process monocalcium phosphate is replaced by crystalline metaphosphate, pyrophosphate, or tricalcium phosphate, or possibly an assemblage of the three, depending upon the kiln temperature and the uniformity of heating the material. The principal reactions and their approximate temperature ranges are\*:

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\* Unpublished data of E. J. Fox of this Bureau.



Calcium orthophosphates—the acid salts, tricalcium phosphate and apatites—are known to be utilizable by animals. Data relating to the suitability of crystalline metaphosphates and pyrophosphates as phos-

TABLE 1.—“Solubility” of pure calcium phosphates in 0.4% HCl  
(Samples were ground to pass a 100-mesh sieve)

COMPOUND			PREPARED BY HEATING—	TOTAL P(P <sub>2</sub> O <sub>5</sub> ) IN	
NO.	NAME	MODIFICATION		SAMPLE	EXTRACT <sup>a</sup>
				Per cent	Per cent of total
2247-a	Calcium metaphosphate	$\beta$	Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> at 600°	30.9 (70.8)	1.1
2247-c	Calcium metaphosphate	$\alpha$	$\beta$ -form at 970°	30.9 (70.8)	0.8
2247-d	Calcium metaphosphate	Vitreous	$\beta$ -form at 1000°	30.9 (70.8)	24.4
1603-a	Dicalcium phosphate	CaHPO <sub>4</sub> <sup>b</sup>	—	22.5 (51.6)	100.0
2234-a	Calcium pyrophosphate	$\gamma$	CaHPO <sub>4</sub> at 600°	24.0 (55.0)	48.9
2234-b	Calcium pyrophosphate	$\beta$	CaHPO <sub>4</sub> at 800°	24.0 (55.0)	21.3
2234-d	Calcium pyrophosphate	$\alpha$	$\beta$ -form at 1200°	24.0 (55.0)	51.1
2262-b	Tricalcium phosphate	$\beta$	$\alpha$ -form° at 1025°	20.2 (46.3)	76.8
2262-a	Tricalcium phosphate	$\alpha$	pptd. material <sup>d</sup> at 1250°	20.2 (46.3)	77.6
2187-b	Hydroxylapatite	—	pptd. material° at 900°	18.4 (42.1)	78.4
2188-b	Silicocarnotite	—	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> , CaCO <sub>3</sub> , and SiO <sub>2</sub> at 1700°	12.7 (29.0)	52.8

<sup>a</sup> Prepared by standard procedure described later.

<sup>b</sup> Hydrated salt that became completely dehydrated on long standing at room temperature.

<sup>c</sup> Inversion of the  $\alpha$ -form yields a coarsely crystalline  $\beta$ -modification, whereas direct heating of the precipitated material at this temperature yields a finely-divided product.

<sup>d</sup> Obtained by evaporating a slurry of phosphoric acid and milk of lime to dryness and igniting the product at 1000°C.

<sup>e</sup> Obtained by hydrolysing a precipitated material  $\left\{ \frac{\text{P}_2\text{O}_5}{\text{CaO}} = 0.78 \right\}$  in an autoclave.

phorus supplements have not as yet been published. Nevertheless, the three classes of calcium phosphates differ markedly in the readiness with which the crystalline forms are attacked by dilute acid (Table 1), so that, if a defluorinated superphosphate be treated with dilute hydrochloric acid by a standardized procedure, the proportion of the phosphorus that is dissolved should afford a measure of the extent of the forementioned reactions and therewith some idea as to the classes of phosphorus compounds present.

*Defluorinated phosphate rock.*—Commercial phosphate rock of domestic origin is essentially a mixture of fluorapatite and silica. When it is heated to 1400°C., or above, in a flowing atmosphere of steam, the fluorine is expelled, and the resulting product is defluorinated phosphate rock. Dependent upon whether the temperature is maintained below or above the melting point of the rock the product has been called *calcined phosphate* (11, 17) or *fused phosphate rock* (4, 6). In either case the principal phos-

phorus compound in the defluorinated material is the high-temperature modification ( $\alpha$ ) of tricalcium phosphate, and therefore the high feeding value found for fused phosphate rock (2, 8, 13) was to be expected.

### MATERIALS

The pure compounds (Table 1) were prepared by the authors and tested for form and homogeneity by microscopic and x-ray diffraction methods.\* Four of the defluorinated superphosphates (Nos. 2263–2265 and 2293b) were prepared in this laboratory for use in animal feeding tests; the other

TABLE 2.—*Screen analyses of thermally defluorinated phosphates, steamed bone meal and Florida waste-pond phosphate*

(Commercial materials sold for feeding purposes except as indicated otherwise)

SAMPLE NO.	MATERIAL	FRACTION THROUGH U. S. STANDARD SIEVE NO. (OPENING IN MM.)					
		12 (1.651)	20 (0.840)	40 (0.420)	60 (0.250)	80 (0.175)	100 (0.149)
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
2221-a	Defluorinated superphosphate	—	100.0	79.6	58.8	46.4	38.6
2220-a	Defluorinated superphosphate	100.0	91.9	72.7	59.8	50.7	44.1
2222-a*	Defluorinated superphosphate	100.0	99.8	99.4	97.2	93.2	88.7
2270	Defluorinated superphosphate	100.0	95.2	78.9	64.3	54.5	42.2
2224	Defluorinated phosphate rock <sup>b</sup>	—	—	—	99.1	98.5	94.8
2248	Steamed bone meal	—	96.2	81.6	62.4	—	38.3
2249	Steamed bone meal	—	89.0	69.5	53.8	—	39.1
2250	Florida waste-pond phosphate	—	64.5	43.1	30.6	—	21.6

\* Experimental material.

<sup>b</sup> Fused phosphate rock.

six were produced by as many commercial concerns. Of the latter samples No. 2270 was prepared in December, 1943, whereas the others were made earlier (in or before May, 1943). For purposes of comparison samples of bone meal, defluorinated phosphate rock, phosphate rock, phosphate slag, vitreous calcium metaphosphate, aluminum phosphate, and some other materials were included in the study. The composition of these samples is given later in connection with other results. Screen analyses of some feed-grade phosphates are given in Table 2.

The defluorinated superphosphates contained 0.05–0.12 per cent of water-soluble  $P_2O_5$  and 0.0–7 per cent of  $P_2O_5$  that was not dissolved by treatment with aqua regia in accordance with the usual practice in fertilizer analysis. Not only does the latter condition necessitate fusion (or some other solubilizing treatment) for a determination of total phosphorus but it also raises the question whether the usual distillation method for fluorine is applicable to such fertilizers. This latter point was tested by re-

\* S. B. Hendricks of this Bureau kindly made the examinations.

determining the fluorine in the material that showed the largest amount of aqua regia-insoluble phosphorus (No. 2229) after fusion of the sample with sodium carbonate, and also after ignition with excess calcium oxide. The results by the three treatments were identical within the precision of the titration, and thereby indicate that direct distillation of the sample is applicable to these materials. In determining the fluorine, the 150-ml. distillate from 0.5 gram of sample was evaporated on the hot plate to a volume of 50 ml. for the titration; otherwise the usual procedure of this laboratory (16) was followed.

#### EXTRACTION OF SAMPLE

Several factors—fineness of sample, digestion temperature, concentration of acid, duration of digestion and weight of sample—that might be expected to influence the quantity of phosphorus brought into solution were investigated to determine suitable conditions for a standard procedure for the preparation of the extract.

*Standard procedure.*—To 1 gram of sample in a 250 ml. Erlenmeyer flask was added 100 ml. of exactly 0.4 per cent (0.1097 *N*) hydrochloric acid, and the mixture was vigorously shaken in the stoppered flask for 30 seconds. The mixture was allowed to digest at 25° for 1 hour with vigorous shaking at intervals of 5 minutes. At the end of the digestion period the undissolved material was filtered off,\* Whatman No. 5 filter paper or its equivalent being used, and washed with distilled water until the volume of filtrate and washings was 200 ml. Where a precipitate formed in the extract, sufficient hydrochloric acid was added to yield a clear solution, which was then made up to 250 ml. The extracts were prepared in duplicate, and aliquots were boiled 2 hours or longer to convert meta- and pyrophosphates to the orthophosphate, and analyzed for phosphorus by the volumetric molybdate method for fertilizer materials (1).

The agreement between single phosphorus determinations on duplicate extracts was satisfactory. The differences of 65 pairs of determinations randomly selected from the results for a variety of materials ranged from 0.00 to 0.50 per cent and averaged 0.10 per cent of  $P_2O_5$ . For the most part the larger differences occurred in the case of the phosphorus-rich pure compounds.

*Fineness of sample.*—The defluorinated phosphates varied considerably with respect to fineness (Table 2). Portions of two defluorinated superphosphates and the defluorinated phosphate rock were ground to different mesh sizes, respectively, and the extracted phosphorus was determined on

\* The difficulty with slow filtration of extracts of steamed bone meal was avoided by the addition of filter paper pulp to the mixture at the beginning of the extraction. The presence of paper pulp influences the amount of phosphorus extracted. In the case of hydroxylapatite, precipitated tricalcium phosphate and waste-pond phosphate, the amounts of extracted phosphorus were increased by 0.4 to 0.6 per cent of  $P_2O_5$  (on sample) with the use of filter paper pulp, whereas in the case of basic slag and silicocarnotite the results were lowered by 0.1–2.6 per cent (silicocarnotite) of  $P_2O_5$ .

these specially prepared samples. The results (Table 3) show only small differences between the extracts of 60- and 100-mesh superphosphates and a considerably larger difference in the case of defluorinated phosphate rock. Although most of the samples used in this study were ground to pass the 100-mesh sieve, it is believed that 80-mesh material is fine enough for routine tests.

*Digestion temperature and acid concentration.*—The variation of extracted phosphorus for three defluorinated superphosphates prepared by commercial concerns with the acid concentration (up to 1.0 per cent of

TABLE 3.—*Effect of fineness of grinding on amount of phosphorus extracted from defluorinated phosphates by standard procedure*

FINENESS SAMPLE GROUND TO PASS	DEFLUORINATED SUPERPHOSPHATE NO. 2220-B		DEFLUORINATED SUPERPHOSPHATE NO. 2222-A	
	-100-MESH MATERIAL IN GROUND SAMPLE	P EXTRACTED	-100-MESH MATERIAL IN GROUND SAMPLE	P EXTRACTED
<i>Sieve No. (opening in mm.)</i>	<i>per cent</i>	<i>per cent of total</i>	<i>per cent</i>	<i>per cent of total</i>
20 (0.840)	47.0	51.6	88.7	63.4
40 (0.420)	62.8	55.8	89.8	63.2
60 (0.250)	84.9	58.1	91.6	63.4
100 (0.149)	100.0	59.7	100.0	63.5

DEFLUORINATED SUPERPHOSPHATE NO. 2270			DEFLUORINATED PHOSPHATE ROCK NO. 2219	
20 (0.840)	49.2	71.9	—	—
40 (0.420)	59.4	73.3	48.8	67.0
60 (0.250)	90.9	76.0	55.0	68.0
80 (0.175)	—	76.4	—	75.0
100 (0.149)	100.0	78.0	100.0	76.8

hydrochloric acid) at room temperature and at 65°C. (other conditions being the same as in the standard procedure) is illustrated graphically in Figure 1. The graph also shows comparable results at room temperature for bone meal, defluorinated phosphate rock, beta tricalcium phosphate and vitreous calcium metaphosphate. Results for one other defluorinated superphosphate (No. 2222-a) prepared by a commercial concern, being very similar to (though somewhat higher than) those for No. 2221-a, are not shown in Figure 1. The indicated effect of digestion temperature on the amounts of phosphorus extracted from these defluorinated superphosphates is small for acid concentration up to about 0.6 per cent, being in most cases least at a concentration of 0.4 per cent. The same is true for defluorinated superphosphates prepared in the laboratory at temperatures above about 700°C. (Figure 2), whereas the results for materials



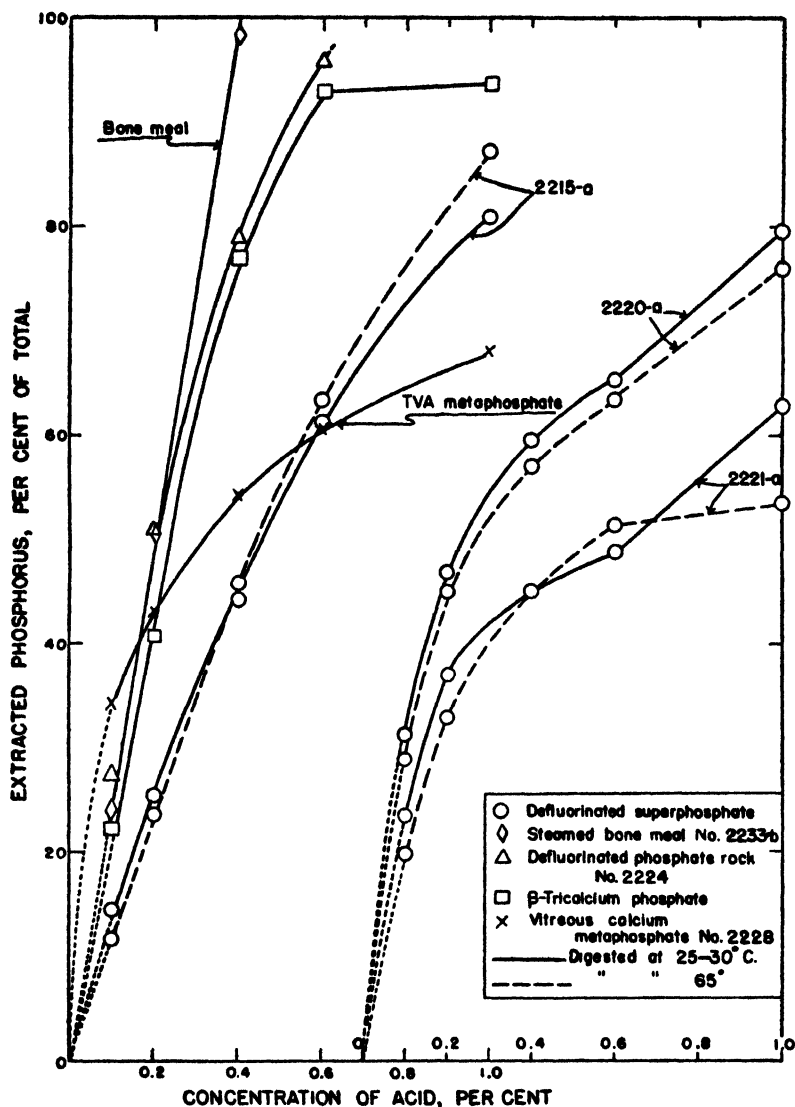


FIG. 1.—EFFECT OF DIGESTION TEMPERATURE AND CONCENTRATION OF HYDROCHLORIC ACID ON AMOUNT OF PHOSPHORUS EXTRACTED FROM COMMERCIALY-PREPARED DEFLUORINATED PHOSPHATES.

prepared at lower temperatures are markedly sensitive to the temperature of digestion. The data obtained on a variety of materials with the use of 0.4 per cent hydrochloric acid are summarized in Table 4, from which it will be observed that with the exception of aluminum phosphate, superphosphate defluorinated at low temperatures, fused phosphate rock,

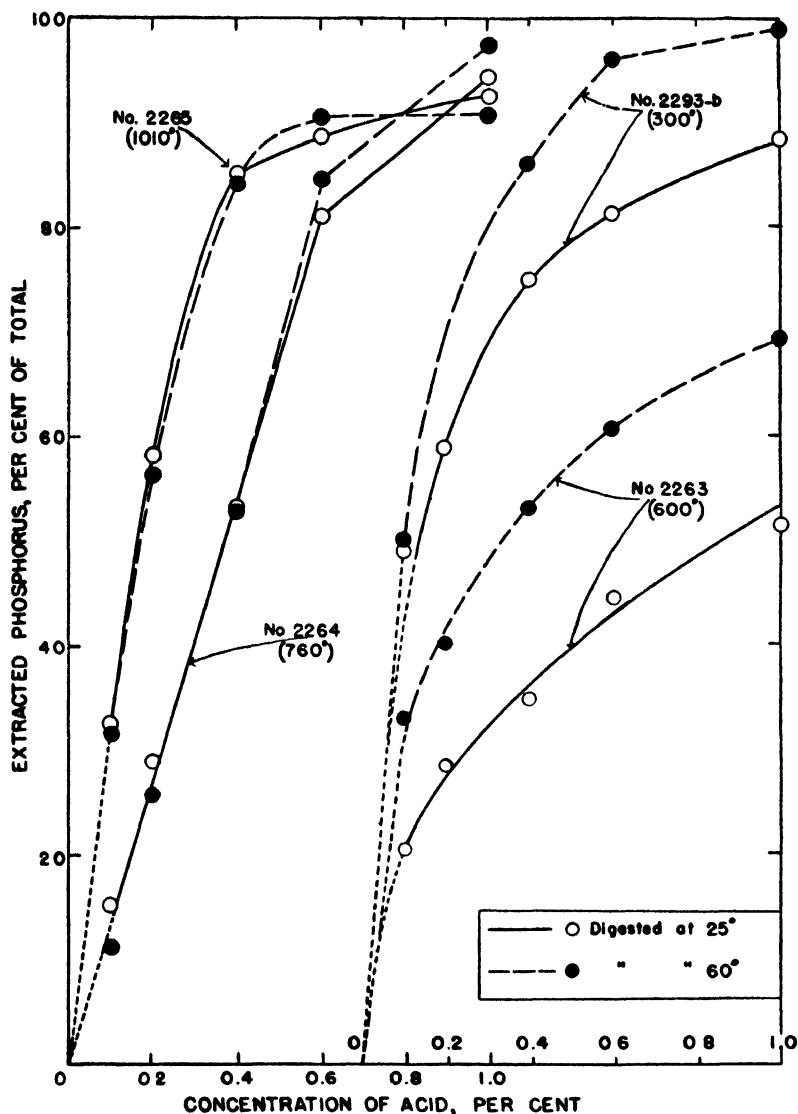


FIG. 2.—EFFECT OF DIGESTION TEMPERATURE AND CONCENTRATION OF HYDROCHLORIC ACID ON AMOUNT OF PHOSPHORUS EXTRACTED FROM LABORATORY PREPARATIONS OF DEFLUORINATED SUPERPHOSPHATE.

and vitreous calcium metaphosphate the results at the two temperatures agree within less than 3 per cent of the total phosphorus.

*Duration of digestion.*—Results for extracted phosphorus obtained on a number of defluorinated phosphates and other materials by varying the period of digestion (Table 5) indicate that with the possible exception of

TABLE 4.—*Effect of digestion temperature on amount of phosphorus extracted by 0.4% HCl*

SAMPLE NO.	SOURCE OR TYPE OF MATERIAL	TOTAL P(P <sub>2</sub> O <sub>5</sub> )	PHOSPHORUS EXTRACTED AT—		INCREASE WITH HIGHER TEMPERATURE
			25°	65°	
Natural Phosphates					
		<i>per cent</i>	<i>per cent of total</i>		<i>per cent of total</i>
2233-b	Steamed bone meal	13.4 (30.8)	98.2	100.0	1.8
2255	Florida waste-pond phosphate	9.7 (22.2)	69.2	70.0	0.8
904	Aluminum phosphate, ignited at 900°	24.2 (55.4)	33.6	56.0	22.4
Pure Compounds					
2262-a	Alpha tricalcium phosphate	20.2 (46.3)	77.6	80.5	2.9
2262-b	Beta tricalcium phosphate	20.2 (46.3)	76.8	78.3	1.5
2268	Beta tricalcium phosphate	19.6 (44.9)	83.6	81.4	-2.2
2234-b	Beta calcium pyrophosphate	24.0 (55.0)	21.3	23.9	2.6
1236	Aluminum phosphate, ignited at 900°	23.6 (54.1)	68.5	79.1	10.6
1237	Ferric phosphate, air-dried	14.3 (32.8)	20.2	19.9	-0.3
Defluorinated Superphosphate					
2293-b*	Laboratory preparation (300°)	9.9 (22.7)	75.0	86.2	11.2
2263*	Laboratory preparation (600°)	10.5 (24.0)	34.8	53.2	18.4
2264*	Laboratory preparation (760°)	11.4 (26.1)	52.9	53.4	0.5
2265*	Laboratory preparation (1010°)	13.1 (30.0)	85.2	84.5	-0.7
2215-a	Commercial preparation, exptl.	11.0 (25.2)	44.4	45.8	1.4
2221-a	Commercial preparation, exptl.	12.6 (28.9)	44.4	45.2	0.8
2220-a	Commercial preparation, exptl.	12.1 (27.7)	59.7	57.2	-2.5
2222-a	Commercial preparation, exptl.	12.7 (29.0)	63.5	63.9	0.4
2270	Commercial preparation, exptl.	12.8 (29.4)	76.4	76.7	0.3
Defluorinated Phosphate Rock and Metaphosphate Glass					
2296	Calcined phosphate <sup>b</sup>	9.0 (20.7)	94.2	96.9	2.7
2224	Fused phosphate rock <sup>c</sup>	12.4 (28.5)	78.8	84.1	5.3
2228	Vitreous calcium metaphosphate <sup>d</sup>	25.7 (58.8)	54.2	95.4	41.2

\* Prepared in this Bureau by heating 100-gram lots of Florida land-pebble superphosphate No. 2261 at the temperature shown in the second column. Nos. 2263-2265 were prepared in a muffle furnace, whereas No. 2293-b was prepared in a tube furnace provided with a flowing atmosphere of steam.

<sup>b</sup> Commercial material.

<sup>c</sup> Produced by the Tennessee Valley Authority.

<sup>d</sup> Fertiliser material (3) produced by the Tennessee Valley Authority.

vitreous calcium metaphosphate little can be gained by digesting longer than 1 hour.

The type of agitation during digestion is of interest in this connection. Although this feature of the method was not studied systematically, the effect of continuous agitation by inversion on a rotating machine was determined on three of the laboratory preparations of defluorinated superphosphate. The results obtained with 1 hour digestion at 25°C. showed

TABLE 5.—*Effect of duration of digestion on amount of phosphorus extracted by 0.4% HCl at 25°C.*

SAMPLE NO.	SOURCE OR TYPE OF MATERIAL <sup>a</sup>	TOTAL P(P <sub>2</sub> O <sub>5</sub> )	PHOSPHORUS IN EXTRACT BY DIGESTING FOR		INCREASE WITH LONGER DIGESTION
			30 MIN.	60 MIN.	
Natural Phosphates and Bone Products					
		<i>per cent</i>	<i>per cent of total</i>		<i>per cent of total</i>
2233-b	Steamed bone meal	13.4 (30.8)	96.2	98.2	2.0
2245	Bone char	16.0 (36.7)	62.9	65.0	2.1
2255	Florida waste-pond phosphate	9.7 (22.2)	69.3	69.2	-0.1
1934	Florida land-pebble phosphate	13.7 (31.3)	55.3	57.4	2.1
Defluorinated Superphosphate					
2293-b	Laboratory preparation (300°)	9.9 (22.7)	72.8	75.0	2.2
2263	Laboratory preparation (600°)	10.5 (24.0)	32.5	34.8	2.8
2264	Laboratory preparation (760°)	11.4 (26.1)	51.8	52.9	1.1
2265	Laboratory preparation (1010°)	13.1 (30.0)	84.5	85.2	0.7
2221-a	Commercial preparation	12.6 (28.9)	43.9 <sup>b</sup>	44.4 <sup>c</sup>	0.5
2222-a	Commercial preparation exptl.	12.7 (29.0)	62.8 <sup>d</sup>	63.2 <sup>e</sup>	0.4
Defluorinated Phosphate Rock and Metaphosphate Glass					
2296	Calcined phosphate	9.0 (20.7)	91.2	94.2	3.0
2224	Fused phosphate rock	12.4 (28.5)	75.6	78.8	3.2
2228	Vitreous calcium metaphosphate	25.7 (58.8)	41.1	54.2	13.1

<sup>a</sup> For further description refer to footnotes to Table 4 and 7.

<sup>b</sup> Result for 15-minute digestion period was 42.3.

<sup>c</sup> Result for 90-minute digestion period was 45.1.

<sup>d</sup> Result for 15-minute digestion period was 61.2.

<sup>e</sup> Result for 90-minute digestion period was 63.3.

increases of 7.2, 17.9, and 1.6 per cent of the total phosphorus, respectively, for materials prepared at 600°, 760°, and 1010°C.

*Weight of sample.*—As might be expected in the case of an empirical method of this type, the amount of extracted phosphorus is in many instances markedly affected by the weight of sample used with a fixed volume of extractant (Table 6). The increases in extracted phosphorus when the size of sample was reduced from 1 to 0.5 gram, range from zero in the case of natural aluminum phosphate to nearly 30 per cent of the total

TABLE 6.—*Effect of weight of sample on amount of phosphorus extracted by 0.4% HCl at 25°C.*

SAMPLE NO.	SOURCE OR TYPE OF MATERIAL <sup>a</sup>	TOTAL P(P <sub>2</sub> O <sub>5</sub> )	PHOSPHORUS IN EXTRACT OF SAMPLE OF		INCREASE WITH SMALLER SAMPLE
			1 GRAM	0.5 GRAM	
Natural Phosphates and Bone Products					
			per cent of total		per cent of total
2233-b	Steamed bone meal	13.4 (30.8)	98.2	98.9	0.7
2245	Bone char	16.0 (36.7)	65.0	94.6	29.6
2255	Florida waste-pond phosphate	9.7 (22.2)	69.2	71.0	1.8
1934	Florida land-pebble phosphate	13.7 (31.3)	57.4	88.4	21.0
904	Aluminum phosphate, ignited at 900°	24.2 (55.4)	33.6	33.6	0.0
Pure Compounds					
2262-a	Alpha tricalcium phosphate	20.2 (46.3)	77.6	94.3	16.7
2234-b	Beta calcium pyrophosphate	24.0 (55.0)	11.7	18.1	6.4
2247-a	Beta calcium metaphosphate	30.9 (70.8)	1.1	1.5	0.4
Defluorinated Superphosphate					
2293-b	Laboratory preparation (300°)	9.9 (22.7)	75.0	83.4	8.4
2263	Laboratory preparation (600°)	10.5 (24.0)	34.8	43.4	8.6
2264	Laboratory preparation (760°)	11.4 (26.1)	52.9	82.4	29.5
2265	Laboratory preparation (1010°)	13.1 (30.0)	85.2	87.0	1.8
2215-a	Commercial preparation, exptl.	11.0 (25.2)	44.4	66.6	22.2
2221-a	Commercial preparation	12.6 (28.9)	44.4	48.4	4.0
2220-a	Commercial preparation	12.1 (27.7)	59.7	67.2	7.5
Defluorinated Phosphate Rock and Metaphosphate Glass					
2296	Calcined phosphate	9.0 (20.7)	94.2	99.1	4.9
2224	Fused phosphate rock	12.4 (28.5)	78.8	96.8	18.0
2228	Vitreous calcium metaphosphate	25.7 (58.8)	54.2	57.1	2.9

<sup>a</sup> For further description refer to footnotes to Tables 1, 4, and 7.

phosphorus in bone char and in a defluorinated superphosphate prepared at 760°C.

It has been rightly suggested\* that 100 ml. of 0.4 per cent of hydrochloric acid will perhaps dissolve only about 0.86 gram of anhydrous tricalcium phosphate (neglecting hydrolytic decomposition of the solution) and that for this reason, as well as on account of the widely variable calcium to phosphorus ratio in the materials subject to test, a larger ex-

\* C. A. Butt, private communication.

tractant to sample ratio would seem desirable. Although the writers are not unfriendly to this view, it may be pointed out that the dissolution of tricalcium phosphate should not be a problem with materials containing less than about 39 per cent of  $P_2O_5$ .

#### RESULTS FOR DIFFERENT PHOSPHATES

The phosphorus extracted with the use of the standard procedure from a variety of phosphate materials is shown in the last column of Table 7, which also contains data relating to the composition of the samples. Comparable data for compounds that are possible constituents of thermally defluorinated phosphates were introduced earlier (Table 1).

On the basis of the results for extracted phosphorus the investigated materials differ widely. In the case of pure crystalline compounds (Table 1) the "solubility" of the metaphosphates is only about 1 per cent and that of the tricalcium phosphates and hydroxylapatite is 77–78 per cent, whereas the pyrophosphates occupy an intermediate position (21–51 per cent). Similarly, the results for commercially prepared materials (Table 7) spread from 100 per cent for a bone meal down to 29.2 per cent for "Accofos," while those for commercially prepared defluorinated superphosphates range from 33.4 per cent for an experimental product produced in the first half of 1943 to 76.4 per cent for a commercial material produced at the close of 1943.

#### APPLICATION TO EVALUATION OF PHOSPHATES FOR MINERAL FEED

The problem of the relative values of different phosphates for mineral feed is one for animal nutrition studies, and the results of any chemical or solubility method must be thoroughly substantiated by feeding tests before the method can have any valid claim to recognition as a measure of availability. In the absence of results of parallel nutrition studies, with which the "solubility" data could be compared, the procedure described above cannot be presented as a method for determining available phosphorus in mineral feeds. Indeed, such a method, if and when it becomes established, may not even involve the use of dilute hydrochloric acid. A few observations bearing on the availability of phosphates by animals may, however, be noted here.

The concentration of hydrochloric acid specified in the standard procedure is well within the range of concentrations (0.1–0.55 per cent) of this acid found in the stomachs of different animals (5), and a phosphate that is only slightly attacked by 0.4 per cent hydrochloric acid, for example crystalline calcium metaphosphate, could scarcely be expected to show favorable results in feeding tests. On the other hand, solubility is apparently not an infallible criterion of availability, for Shelling and Asher (19) found no evidence of assimilation of phosphate ingested in the form of

**TABLE 7.—Composition of feed-grade phosphates and related materials**  
(Samples were ground to pass 100-mesh sieve, unless indicated otherwise)

SAMPLE NO.	SOURCE OR TYPE OF MATERIAL	F	CaO (Al <sub>2</sub> O <sub>3</sub> ) (Fe <sub>2</sub> O <sub>3</sub> )	SO <sub>3</sub>	TOTAL P(F <sub>2</sub> O <sub>5</sub> ) IN	
					SAMPLE	ACID EXTRACT <sup>a</sup>
		per cent	per cent	per cent	per cent	per cent of total
<b>Bone Products</b>						
2233-b <sup>b</sup>	Steamed bone meal	0.06	40.8	—	13.4 (30.8)	98.2
2248 <sup>b</sup>	Steamed bone meal	0.03	37.0	—	12.2 (27.9)	100.0
2245	Bone char <sup>c</sup>	0.09	52.4	—	16.0 (36.7)	65.0
<b>Defluorinated Superphosphate</b>						
2229	Commercial preparation, exptl.	0.40	39.6	28.2	11.8 (27.0)	33.4
2263 <sup>d</sup>	Laboratory preparation (600°)	0.06	34.0	32.8	10.5 (24.0)	34.8
2215-a	Commercial preparation, exptl.	0.04	37.6	28.7	11.0 (25.2)	44.4
2221-a	Commercial preparation, exptl.	0.01	37.7	20.8	12.6 (28.9)	45.1
2264 <sup>d</sup>	Laboratory preparation (760°)	0.01	37.2	26.1	11.4 (26.1)	52.9
2220-a	Commercial preparation	0.24	42.2	20.4	12.1 (27.7)	59.7
2222-a	Commercial preparation, exptl.	0.01	40.9	19.2	12.7 (29.0)	63.5
2293-b <sup>d</sup>	Laboratory preparation (800°)	0.21	31.5	33.5	9.9 (22.7)	75.0
2270	Commercial preparation	0.09	44.1	19.7	12.8 (29.4)	76.4
2265 <sup>d</sup>	Laboratory preparation (1010°)	0.02	42.8	15.6	13.1 (30.0)	85.2
<b>Defluorinated Phosphate Rock</b>						
2296 <sup>b</sup>	Calcined phosphate	0.02	30.4	—	9.0 (20.7)	94.2
2224 <sup>b</sup>	Fused phosphate Rock <sup>e</sup>	0.02	40.4	—	12.4 (28.5)	78.8
2252	"Accofos"	2.18	—	—	13.7 (31.5)	29.2
<b>Other Furnace Products</b>						
2282	Slag <sup>f</sup>	0.04	38.1 <sup>g</sup>	—	8.5(19.4)	89.3
2227	Slag <sup>h</sup>	0.01	35.9 <sup>i</sup>	—	13.5 (31.3)	68.1
2228	Vitreous calcium metaphosphate <sup>j</sup>	0.39	24.6	—	25.7 (58.8)	54.2
<b>Phosphate Rock</b>						
2255	Florida waste-pond phosphate <sup>k</sup>	1.78	24.6	—	9.7 (22.2)	69.2
985	Curacao Island	0.70	50.0	0.6	16.8 (38.4)	67.3
1934	Florida land pebble	3.75	45.2	0.7	13.7 (31.1)	57.4
1936	Tennessee brown rock	3.52	44.6	1.3	14.2 (32.5)	56.0
<b>Miscellaneous Products</b>						
2261	Florida land-pebble superphosphate	1.46	28.8	30.8	9.1 (20.8)	94.6
2251	Pofos <sup>l</sup>	1.54	34.0	—	7.7 (17.7)	90.0
1237	Ferrio phosphate, synthetic	—	(41.8)	—	14.3 (32.8)	20.2
904	Aluminum phosphate, natural	0.05	(28.6)	—	18.5 (42.3)	2.0
1236	Aluminum phosphate, synthetic	—	(26.7)	—	15.9 (36.5)	95.5

<sup>a</sup> Prepared by the standard procedure.

<sup>b</sup> Sample was ground to pass 80-mesh sieve.

<sup>c</sup> Spent bone black from a sugar refinery.

<sup>d</sup> Prepared in this Bureau by heating 100-gram lots of Florida land-pebble superphosphate No. 2261 at the temperature shown in the second column. Nos. 2263-2265 were prepared in a muffle furnace, whereas No. 2293-b was prepared in a tube furnace provided with a flowing atmosphere of steam.

<sup>e</sup> Prepared by the fusion process (6) at plant of Tennessee Valley Authority.

<sup>f</sup> Produced experimentally by Tennessee Valley Authority by calcining ferrophosphorus with limestone in a rotary kiln.

<sup>g</sup> Contains 35.8% of Fe<sub>2</sub>O<sub>3</sub>.

<sup>h</sup> Produced experimentally by a commercial concern.

<sup>i</sup> Contains 2.42% Al<sub>2</sub>O<sub>3</sub>, 5.16% Fe<sub>2</sub>O<sub>3</sub> (total Fe), 3.32% MnO and 9.55% MgO.

<sup>j</sup> Fertiliser material (3) produced by Tennessee Valley Authority.

<sup>k</sup> Sold under the trade name "Sea-Coll" for use in animal feeds. Waste-pond phosphate has also been marketed under the names, Colloidal Phosphate, Collophos, Mineral Colloids, Mim-Coll, Phosphate Colloids, Cal-Phos, PhosCalOids, Vitaloid Phosphate, Lonfosco and Colimephos.

<sup>l</sup> Trade name for a material sold as "partially defluorinated phosphate" for use in poultry feeding.

water-soluble sodium metaphosphate. Availability results obtained in rat feeding experiments\* with defluorinated superphosphates 2220-a, 2221-a, and 2222-a place these materials in the same order as do the "solubility" data (Table 7), and according to nutrition studies on rats by Fraser and others (8) the availabilities of defluorinated (fused) phosphate rock, crude vitreous calcium metaphosphate, and pure vitreous calcium metaphosphate, respectively, are 96, 73, and 49 relative to sodium orthophosphate as 100, in comparison with the sequence 80, 55, and 25 obtained from the "solubility" data for very similar samples and taking steamed bone meal as the standard of reference.

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\* Private communication from N. R. Ellis, Bureau of Animal Industry, Beltsville, Maryland



DETERMINATION OF AMINOAZOXYLENE IN D&C  
RED NO. 18

By O. L. EVENSON (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

D&C Red No. 18 (Oil Red OS) 1-Xylylazoxylazo-2-naphthol, is listed by the Food and Drug Administration among the coal-tar colors certifiable for use in drugs and cosmetics.<sup>1</sup> In the preparation of this color another azo dye, aminoazoxylene, is used as an intermediate.

The proposed method for the determination of this intermediate includes a separation of aminoazoxylene from a petroleum benzine solution of D&C Red No. 18 by extraction with an acidic water-alcohol mixture followed by removal of the intermediate from this mixture by steam distillation.

## METHOD

## REAGENTS

- (a) *Ethyl alcohol*.—95 %.
- (b) *Petroleum benzine*.—30°–65°C. b.p.
- (c) *Acidic water-alcohol extraction mixture*.—To 600 ml. of distilled water add 200 ml. of H<sub>2</sub>SO<sub>4</sub>, cool, add 200 ml. of ethyl alcohol, and mix.
- (d) *Standard solution of aminoazoxylene*.—Dissolve 50 mg. of *p*-amino-azoxylene (m.p. 143°–44°C.) in 500 ml. of 95 % ethyl alcohol.

## APPARATUS

For steam distillation use a 500 ml. flask which has a ground-glass joint and is equipped with a distilling head, the steam inlet tube of which reaches nearly to the bottom of the flask. Numerous fine holes at the tip of the tube permit the steam to spread at the bottom of the flask. Connect distilling head with a vertically placed, 20-inch, straight tube condenser, using ground glass joints.

## DETERMINATION

Dissolve 1 gram of D&C Red No. 18 in 100 ml. of CHCl<sub>3</sub>. Transfer 5 ml. of this solution to a 500 ml. separatory funnel containing 250 ml. of petroleum benzine. Extract the aminoazoxylene by shaking vigorously with several 20 ml. portions of the acidic water-alcohol mixture (four 20 ml. portions are usually sufficient). Transfer the extracts to a 250 ml. volumetric flask. (If less than 0.1 % of intermediate is present in the dye, a 100 ml. flask should be used.) Dilute to the mark and mix. Transfer an aliquot containing approximately 0.1 mg. of aminoazoxylene to the steam distillation flask. Cool in an ice bath and make just alkaline to litmus with Na<sub>2</sub>CO<sub>3</sub>, added in small quantities. Adjust the volume to about 50 ml. and add about 20 grams of NaCl. Insert the distilling head and connect with the condenser. Heat nearly to boiling and connect with a generator which delivers steam without bumping. Distil slowly at first (too rapid generation of steam or boiling of the contents in the distillation flask may result in loss of the intermediate). Collect the distillate in a 100 ml. flask. When about 85 ml. has been collected, discontinue the distillation, disconnect, and wash the condenser with about 15 ml. of 95 % alcohol and drain into the

<sup>1</sup> S.R.A., F.D.C. 3, Food and Drug Administration, Federal Security Agency.

receiving flask. Transfer the distillate to a 250 ml. separatory funnel and extract the intermediate with 3 portions (30, 25, and 20 ml.) of ethyl ether. Drain the extracts into a wide Nessler or suitable test tube and remove the solvent at room temperature with the aid of a gentle stream of air washed by passage through  $\text{H}_2\text{SO}_4$ . Dissolve the residue in 20 ml. of 95% alcohol and compare with standards made to contain 0.05–0.15 mg. of aminoazoxylene in the same volume of solvent.

Known quantities of aminoazoxylene were added to 50 mg. of D&C Red No. 18, dissolved in petroleum benzene and previously shaken with portions of the acidic water-alcohol mixture to remove any intermediate

TABLE 1.—*Recovery data*

SAMPLE	AMINOAZOXYLENE	
	ADDED mg.	RECOVERED per cent
1	0.10	90
2	0.25	88
3	0.50	95
4	1.00	90
5	1.25	93
6	1.50	88
7	2.00	90
8	2.50	93

Average 91

originally present, and recoveries were determined. The results in Table 1 indicate a recovery of approximately 90 per cent.

#### DISCUSSION

The process of steam distillation introduced in the method accounts in some measure for the low recoveries shown in Table 1, but this procedure was necessary to obtain a product comparable in shade to the standards. Aminoazoxylene distills readily, all but a small proportion coming over in the first few minutes. The distillate may sometimes be nearly colorless due to lowering of the *pH* by the carbon dioxide that comes over with the steam. Aminoazoxylene when present in low concentrations in water is nearly colorless at *pH* 3.5. At about *pH* 5, however, it is yellow and at *pH* 2.5 turns red. The addition of sodium chloride lowers the vapor pressure of the aqueous solution, and it also tends to decrease the solubility of the aminoazoxylene and promote its volatilization with steam. Xylidines, if present, also come over with steam, but they do not interfere in the colorimetric test.

#### SUMMARY

A method is proposed for the determination of aminoazoxylene in D&C Red No. 18.

Typical data, indicating 90 per cent recoveries, are given.

## IDENTIFICATION OF THIOGLYCOLIC ACID

By J. H. JONES (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

Thioglycolic acid is a component of many solutions for cold permanent waving. Hoshall<sup>1</sup> and Shupe<sup>2</sup> have described several color reactions and precipitation tests for the identification of this acid. However, most of these reactions are not specific for thioglycolic acid but are given also by other thiols. In addition, sulfites, thiosulfates, and sulfides, which may be present in waving preparations, obscure the precipitation tests.

A more specific identification may be obtained by the conversion of thioglycolic acid to dithiodiglycolic acid and identification of the latter. In neutral or acid solution thioglycolic acid, like other thiols, is oxidized by iodine to the corresponding disulfide. Dithiodiglycolic acid is very soluble in water but may be partially extracted from an acidified solution with ether. Evaporation of the ether gives the solid dithiodiglycolic acid, which may be recrystallized and identified by its melting point. The neutralization equivalent of dithiodiglycolic acid can be determined accurately and may be used to confirm the identity of the derivative.

The following procedure is tentatively suggested for the utilization of these properties in the identification of thioglycolic acid in permanent wave solutions.

## TENTATIVE METHOD

## REAGENTS

*Iodine solutions.*—Approximately 1*N* and approximately 0.1 *N*.

*Sodium bisulfite solution.*—5%.

*Benzene-ethyl acetate solvent.*—Mix 9 volumes of benzene and 1 volume of anhydrous ethyl acetate.

## PROCEDURE

Place an aliquot of the solution containing 0.2–1.0 gram of thioglycolic acid (10 ml. is usually sufficient for permanent wave solutions) in a separatory funnel and add HCl until the solution is slightly acid. Use starch solution as an indicator and oxidize the sulfur compounds with approximately 1 *N* iodine. Decolorize by dropwise addition of the bisulfite solution, filter if any water-insoluble compounds have been produced, and adjust to the exact starch-iodine end point with 0.1 *N* iodine. Add concentrated HCl, equal to about 50% of the volume of the solution at this point, extract with 20 ml. of CHCl<sub>3</sub>, and discard the CHCl<sub>3</sub> layer. Extract with three portions of ethyl ether (50, 25, and 25 ml.), combine the ether extracts, and wash with two 10 ml. portions of water. (A small amount of iodine in the ether layer at this point does no harm.) Filter the ether through a pledget of cotton, placed in a long stem funnel, and evaporate to dryness on a steam bath. Dissolve the residue in the minimum amount of boiling benzene-ethyl acetate solvent, filter, cool, and let stand until the crystals form. (It may be necessary to scratch the sides of the container

<sup>1</sup> *This Journal*, 23, 727 (1940).

<sup>2</sup> Private communication.

vigorously to induce crystallization.) Filter, wash the white precipitate with cold benzene, dry, and determine the melting point. (Melting points listed by Mullikan<sup>3</sup> for dithiodiglycolic acid are 100° and 107°–108°C.)

To determine the equivalent weight of the acid, dissolve an accurately weighed quantity in a small quantity of water, add 2–3 drops of phenolphthalein indicator, and titrate with standard NaOH. (The theoretical equivalent weight is 91.2.)

Dithiodiglycolic acid is a white, non-hygroscopic, odorless solid. It is very soluble in water, alcohol, ethyl ether, and ethyl acetate, but only slightly soluble in benzene, chloroform, and petroleum benzine.

Attempts to recrystallize dithiodiglycolic acid by the procedures proposed in the literature often failed to give a pure product. This was particularly true if only a small amount of material was available. The proposed benzene-ethyl acetate mixture appears to be a suitable solvent when the recrystallization of small quantities of the acid is involved. Derivatives prepared from commercial permanent wave solutions by the above method usually melted above 100°C. after a single recrystallization. When sufficient material for several recrystallizations was available, a product whose melting point agreed with the higher values reported in the literature could be obtained. Typical results for such a series of recrystallizations are shown in Table 1.

TABLE 1.—*Purification of dithiodiglycolic acid*

NUMBER OF RECRYSTALLIZATIONS	MELTING POINT  °C.	EQUIVALENT WEIGHT
1	98–102	94.0
2	104–105	92.4
3	105–106	92.2
4	105–106 (106–107)*	91.4

\* Capillary tube method. All other melting points determined on a melting point block.

Either macro or semi-micro (25–50 mg.) samples may be used for the equivalent weight determinations.

The procedure described has been successfully used to identify thioglycolic acid in a number of permanent wave solutions. No other sulfur compounds proposed for use in such preparations give after oxidation with iodine a product soluble in water and extractable with ether. The single extraction with chloroform and recrystallization from benzene-ethyl acetate usually eliminate the small quantity of oil or fatty acid present. If a large quantity of oil is present, several extractions with chloroform must be made.

The proposed procedure has not been applied to depilatories, but with

<sup>3</sup> "Identification of Pure Organic Compounds," Vol. IV, p. 108. John Wiley and Sons, New York (1922).

suitable modifications it could undoubtedly be used to identify thioglycolates in this type of preparation.

The proposed extraction is not quantitative, but sufficient dithiodiglycolic acid for identification can be obtained from 0.2 gram of thioglycolic acid. Experiments are in progress to determine whether the extraction can be made quantitative by suitable modifications. If quantitative extraction could be obtained, it would provide a method for the determination of thioglycolic acid in the presence of other reducing compounds.

## SPECTROPHOTOMETRIC ANALYSIS OF COAL-TAR COLORS I. EXT D&C YELLOW NO. 5

By G. R. CLARK and S. H. NEWBURGER (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

For some of the colors certifiable under the Coal-Tar Color Regulations,<sup>1</sup> the most satisfactory way of estimating the pure dye content has been to calculate it from a determination of the nitrogen in the dye. The objection to such a method is that the presence of any nitrogenous material as an impurity will lead to erroneous results. Spectrophotometric methods of analysis have therefore been investigated for some of these colors. The results indicate that such methods are of adequate precision for determining the pure dye, and that they also serve to identify the colors.

In these investigations samples sufficiently pure to serve as standards were prepared, and spectrophotometric data were obtained from solutions of these samples. From these data the applicability of Beer's law was checked; absorption peaks were determined; and extinction ratios<sup>2</sup> were calculated, at suitable wave lengths, to aid in the identification of the colors.

All optical measurements were made with a General Electric recording spectrophotometer equipped with slit adjustments for an 8 millimicron wave length band.

Results obtained in the investigation of Ext D&C Yellow No. 5 (Hansa Yellow) are reported here. Data for other colors will be presented in future numbers of *This Journal*.

### EXPERIMENTAL

*Preparation of Standard Sample.*—3-Nitro-4-aminotoluene (m.p. 117°C.) was diazotized and coupled with acetoacetanilid (m.p. 83°C.) in alkaline

<sup>1</sup> S.R.A.F.D.C. 3, U. S. Food and Drug Administration, Washington, D. C.

<sup>2</sup> Technical Bulletin No. 310, U. S. Department of Agriculture, p. 21. June, 1932.

solution. The product was crystallized twice from 1,4 dioxan and melted at 258°C. (literature<sup>3</sup> gives 256°C.).

A portion of this material, recrystallized from 2-nitropropane, showed no change in melting point. Spectrophotometrically, an examination of chloroform solutions showed no difference between this material and that obtained from 1,4 dioxan. The substance crystallized from 1,4 dioxan was therefore considered sufficiently pure to serve as a standard.

*Preparation of Solutions.*—A 20.04 mg. portion of dye was weighed on a semi microbalance, sensitive to 0.02 mg., and dissolved by warming with 80 ml. of chloroform. The solution was cooled to room temperature, trans-

TABLE 1.—*Extinction values of chloroform solutions of Ext D & C Yellow No. 5*

CURVE NO. (CHART 1)	CONCENTRATION	$E_{413\text{ m}\mu}$	$E_{413\text{ m}\mu}$
			CONCENTRATION
	<i>mg./liter</i>		
1	2.50	0.157	.0628
2	5.01	0.317	.0633
3	10.02	0.630	.0629
4	20.04	1.263	.0630
			Av. .0630

ferred to a 100 ml. volumetric flask, and made to volume with chloroform. Aliquot portions of these solutions were diluted with chloroform to the concentrations shown in Table 1. All solutions were made to volume at the temperature of the room in which the optical measurements were made.

*Spectrophotometric Data.*—The chloroform solution of Ext D&C Yellow No. 5 shows an absorption peak at  $413 \pm 2\text{ m}\mu$ . (All wave lengths were corrected to  $\pm 2\text{ m}\mu$  with the aid of didymium glasses tested by the National Bureau of Standards; cf. footnote to Charts 1–3, inclusive.)

The ratio of the extinction values at 410 and 430  $\text{m}\mu$  was measured for use as an aid in the identification of the color. This ratio is  $E_{410\text{m}\mu}/E_{430\text{m}\mu} = 1.23 \pm 0.02$ .

For the extinction curves and the tabulated data, see Chart 1 and Table 1.

## DISCUSSION

The ratios of extinction to concentration in Table 1 indicate that at 413  $\text{m}\mu$  deviations from Beer's law are less than 1 per cent for chloroform

<sup>3</sup> Fierz, David, H. E., and Ziegler, E., *Helv. Chim. Acta*, 11, 776 (1928).

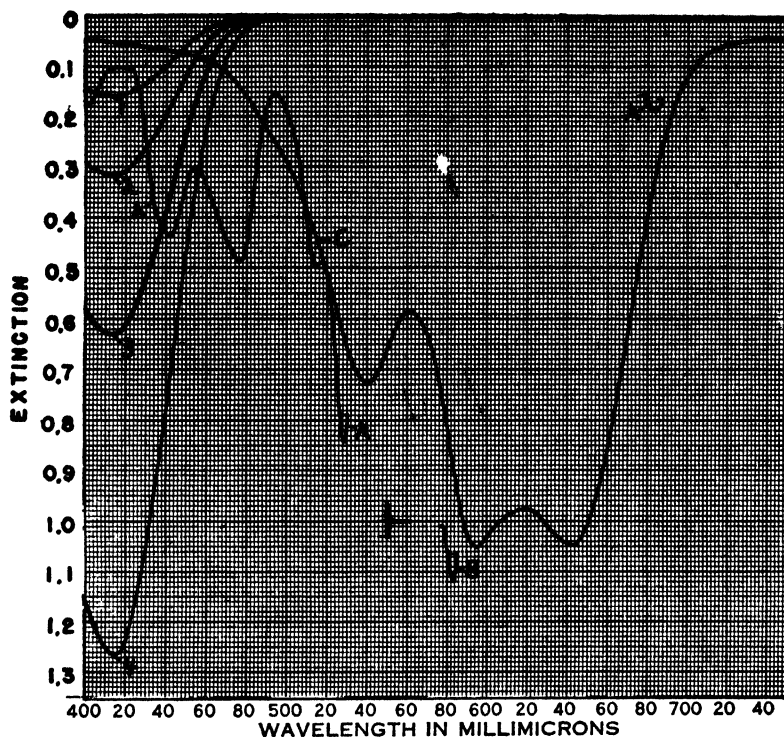


CHART 1.—PURE EXT D&C YELLOW NO. 5 IN CHLOROFORM.

Curve 1—2.5 mg./liter.

Curve 2—5.01 mg./liter.

Curve 3—10.02 mg./liter.

Curve 4—20.04 mg./liter.

Cells—1 cm.

A = Corning Didymium Glass 512, 6.0 mm.

(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8  $m\mu$ )

B = Corning Didymium Glass 592, 4.02 mm.

(Absorption peak at 583.7  $m\mu$ .)

C = Signal Lunar White Glass-H-6946236.

solutions of Ext D&C Yellow No. 5 containing 2.5–20 mg. of color per liter. The pure dye content of a sample of this color can therefore be calculated from spectrophotometric data on its chloroform solution by comparison with a standard solution of known concentration.

#### APPLICATION TO COMMERCIAL SAMPLES

Two samples of certified Ext D&C Yellow No. 5 were analyzed spectrophotometrically. Weighed samples were dissolved in 100 ml. of chloroform, and extinction measurements were made on suitably diluted aliquots. The curves are shown on Chart 2, and the data are presented in Table 2.

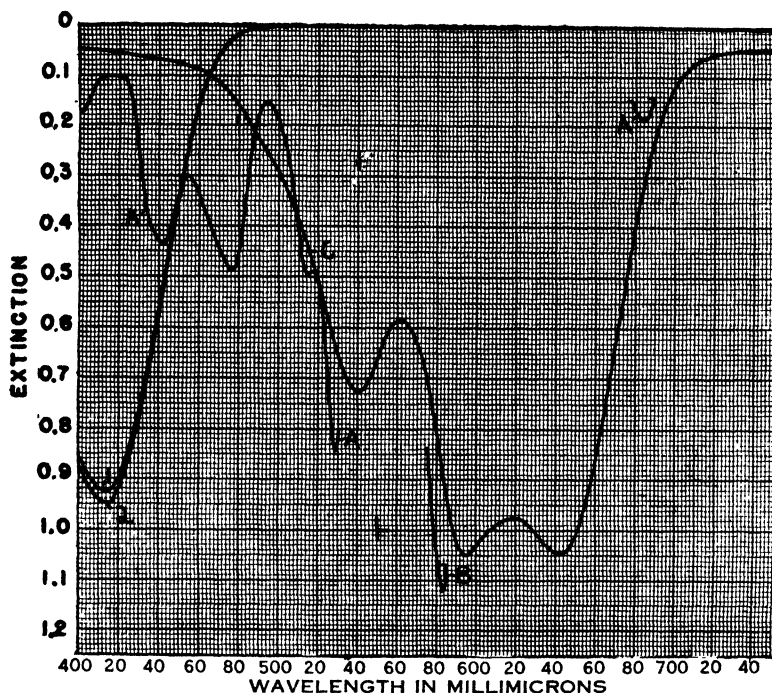


CHART 2.—CERTIFIED SAMPLES EXT. D&C YELLOW NO. 5 IN CHLOROFORM.

Curve 1 = Sample 1—15.00 mg./liter.

Curve 2 = Sample 2—15.17 mg./liter.

Cells—1 cm.

A = Corning Didymium Glass 512, 6.0 mm.

(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 mμ.)

B = Corning Didymium Glass 592, 4.02 mm.

(Absorption peak at 583.7 mμ.)

C = Signal Lunar White Glass H—6946236.

TABLE 2.—Analysis of certified samples of Ext D&C Yellow No. 5

SAMPLE NO.	M. P.	CONCENTRATION	E <sub>413 mμ</sub>	DYE* SPECTROPHOTO- METRICALLY	DYE FROM N <sub>2</sub> CONTENT
	°C.	mg./liter		per cent	per cent
1	257	15.00	0.925	98.0	98.0
2	258	15.17	0.947	99.0	98.5

\* The dye content was calculated by using .063 (Table 1) as the extinction value for 1 mg./liter of pure Ext D&C Yellow No. 5.

The pure dye content of lakes made by extending Ext D&C Yellow No. 5 on insoluble substrata may also be determined by the spectrophotometric method. The color is dissolved by refluxing the sample with chloro-



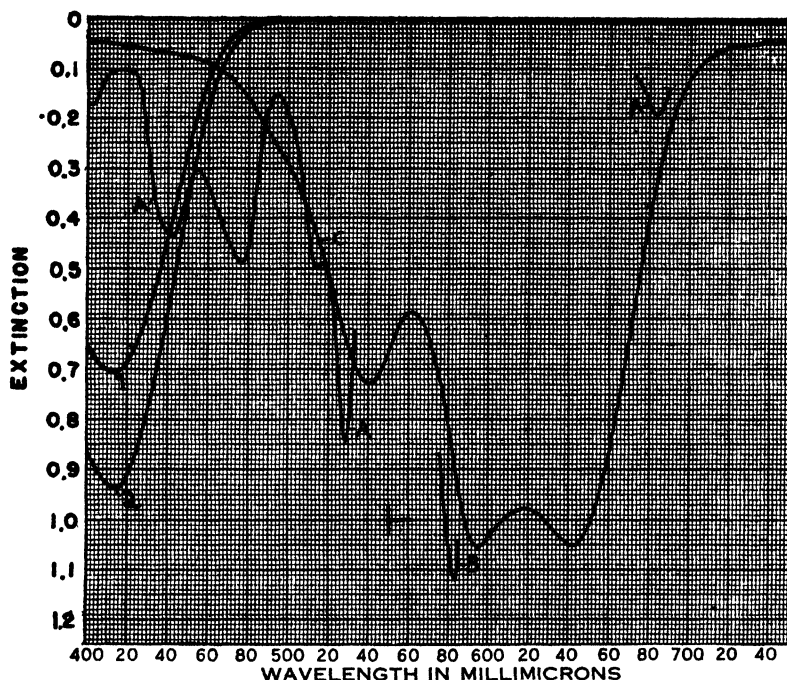


CHART 3.—EXT. D&C YELLOW No. 5 (IN CHLOROFORM)

EXTRACTED FROM CERTIFIED LAKES.

Curve 1 = Sample 1—concentration of lake—23.6 mg./liter.

Curve 2 = Sample 2—concentration of lake—201 mg./liter.

Cells—1 cm.

A = Corning Didymium Glass 512, 6.0 mm.

(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 mμ.)

B = Corning Didymium Glass 592, 4.02 mm.

(Absorption peak at 583.7 mμ.)

C = Signal Lunar White Glass—H-6946236.

form, filtering the solution to remove the substratum, and diluting to suitable concentration. The pure dye content is then determined as described above.

TABLE 3.—Analysis of certified lakes of Ext D&C Yellow No. 5

SAMPLE NO.	QUANTITY OR LAKE TAKEN	E <sub>413</sub> mμ	DYE SPECTROPHOTO- METRICALLY	DYE FROM N <sub>2</sub> CONTENT
1*	mg./liter 23.6	0.707	per cent 47.6	per cent 47.0
2†	201	0.935	7.4	7.35

\* Substratum—Alumina and talc.

† Substratum—Gloss white.

Application of this method to two samples of certified lakes is shown in Chart 3 and Table 3.

As shown in Tables 2 and 3, the pure dye content for both primary colors and lakes as determined by spectrophotometric measurements agrees with that calculated from the determination of nitrogen.

#### SUMMARY

Spectrophotometric data for chloroform solutions of purified Ext D&C Yellow No. 5 are presented. Beer's law is shown to be applicable; the absorption peak is at 413  $m\mu$ , and the extinction ratio  $E_{410\text{ }m\mu}/E_{480\text{ }m\mu}$  is  $1.23 \pm 0.02$ .

Application is made of these data to the determination of pure dye in commercial samples of the color.

Typical results are given.

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#### REPORT ON FERTILIZERS

By G. S. FRAPS (Agricultural Experiment Station,  
College Station, Texas), *Referee*

Very little referee work has been done during the last year. W. H. Ross, Associate Referee on Phosphoric Acid, has carried out some cooperative pot tests to determine the availability of nitrogen in phosphates, with different degrees of ammonification, and H. R. Allen, Associate Referee on Acid- and Base-forming Quality, has conducted work on his subject. W. Y. Gary, Associate Referee on Copper and Zinc, is now in the Navy. All the work has been decreased on account of necessary attention to other work relative to the war effort.

A number of recommendations\* adopted as official, first action, in 1941 should now receive attention for final action. A few other recommendations are being made.

#### RECOMMENDATION FOR FINAL ACTION AS OFFICIAL

The following methods have been adopted as official, first action (*This Journal*, 25, 47-50, unless otherwise stated) as recommended by the Referee (*Ibid.*, 314 chiefly) and are recommended for adoption as official, final action. The references given are to the paragraph and page in *This Journal*, Vol. 25, for the committee action in 1941.

(1) That the slotted single-tube sampler, the slotted double-tube sampler, and slotted tube and rod sampler (*Ibid.*, 24, 501), all with solid pointed ends, be adopted for sampling fertilizer (1, p. 20) (official, final action).

(2) That par. 41 (a) and (b), p. 31, of the official potash method, be changed to provide for the use of factor weight by adding "or the factor

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\* For report of Subcommittee A and action by the Association, see *This Journal*, 27, 45 (1944).

weight 2.425 g" after "2.5 g" in the first and second lines, respectively, and that to 42 (a) there be added, "If the factor weight and a 50 ml aliquot (containing 0.485 g sample) are used, multiply weight by 40 to get per cent  $K_2O$ " (official, final action).

(3) That the official method for the determination of water-insoluble organic nitrogen in 34, p. 29 be deleted (final action) (10, p. 48).

(4) That for section (b) of the official method for the determination of nitrate nitrogen (31, p. 28), the following directions be substituted, "(b) Determine water-insoluble organic nitrogen as directed under 35 but use 2.5 g of the mixed fertilizer" (official, final action).

(5) That the heading "Total Nitrogen," preceding pars. 24, 25, 26, and 27 be changed to read, "Total Nitrogen in Mixed Fertilizers" (official, final action).

(6) That the words "in mixed fertilizer" be added to the heading, "Nitrate Nitrogen" preceding par. 31, p. 28 (official, final action).

(7) That in line 6 of par. 60, p. 38, after the words "for unmixed nitrate salts," the words "or for mixed fertilizers containing considerable nitrate nitrogen" be added (official, final action).

(8) That the long volumetric method for the determination of copper, adopted as tentative in 1941, and published in *This Journal*, 24, 67, be adopted as official (final action).

(9) That the method for the determination of citrate-insoluble phosphoric acid in non-acidulated samples other than basic slag (16 (b), p. 24) be changed to read as follows:

*Dicalcium phosphate*.—Place 1 g of the sample on a 9 cm qualitative filter paper in a 250 ml Erlenmeyer flask and proceed as directed in 16 (a). (Official, final action) *This Journal*, 25, 314, also p. 49.

(10) That the statement "(Not applicable to samples containing free ammonia or compounds other than water that are volatilized at the temperature of drying)," be added to the method for the determination of moisture by drying, 4, p. 20 (official, final action).

(11) That to Chapter II, the following paragraph be added: "*Graduated flasks*.—The maximum diameter of graduated flasks at the capacity mark should not exceed the limits set by National Bureau of Standards (Circ. 9, 1916), namely, 13 mm for 200 ml flasks, 15 mm for 250 ml flasks, or 18 mm for 500 ml flasks" (official, final action).

(12) That in the official Devarda method (30, p. 27) the quantity "0.5 g" be changed to "0.35 or 0.5 g"; that the last paragraph be changed to read, "In the analysis of nitrate salts, dissolve 3.5 or 5.0 g in  $H_2O$ , make up to 250 ml, and use 25 ml"; and that the same sentence be added to the ferrous sulfate-zinc-soda method for the same determination (29, p. 27) (official, final action).

(13) That the following sentence be added to the method for the prepa-

ration of sodium or potassium hydroxide solution (10 (b), p. 22): "Burets in constant use are likely to become so corroded as to increase their capacity, and therefore should be tested at least once a year (official, final action).

(14) That the following clarifying paragraph be added to the method for the determination of potash as 41 (e), p. 31:

*Nitrate of potash or nitrate of potash and soda.*—If impure, proceed as directed in (a); if sufficiently pure, proceed as directed for potash salts (b), except to evaporate an aliquot to dryness in a porcelain dish with 2 ml of HCl (if Pt dish is used, add  $\text{H}_2\text{SO}_4$  instead) and to take up with  $\text{H}_2\text{O}$  and a few drops of HCl, before adding the Pt soln (official, final action).

(15) That Method III, *Volumetric Modification of the Method for Acid-soluble Magnesium*, 54, p. 36, adopted as official (first action), *This Journal*, 24, 47, be adopted as official, final action.

The bismuthate volumetric method for manganese is more easily carried out and is preferred to the iodate method, and both methods are not needed, according to the report of the associate referee. The Referee therefore recommends:

(16) That the bismuthate volumetric method for manganese, *This Journal*, 24, 69, adopted as official (first action), *Ibid.*, 25, 49, be adopted as official, final action.

(17) That the tentative volumetric periodate method for manganese, 56, 57, p. 37, be deleted (final action).

(18) That the volumetric method for manganese, 58, p. 37, adopted as official (first action), *This Journal*, 24, 47, and corrected editorially, *Ibid.*, 25, 49, 79, be adopted as official, final action.

#### RECOMMENDATION FOR OFFICIAL ADOPTION, FIRST ACTION

The associate referee reports that Method I for acid-soluble manganese is accurate but long, is probably seldom used, has practically been replaced by Method II, and is no longer needed. The Referee therefore recommends:

(1) That Method I, official for acid-soluble magnesium, 52, p. 35, be deleted, but that such portions as are referred to in Method II, 53, p. 36, be incorporated in Method II when revised (official, first action).

#### RECOMMENDATION FOR ADOPTION AS TENTATIVE

That titration with a glass electrode be adopted as tentative in the method for acid- and base-forming quality, as recommended by the associate referee, as follows:

(1) Proceed as directed in 60, p. 38, through the addition of 50 ml of water and 30 ml of normal HCl and digestion on a hot plate or steam bath for 1 hour. Cool to room temperature and without filtering titrate the solution in the 150 ml beaker

with 0.5 *N* NaOH to pH 4.3, using a glass electrode apparatus or other standard means of electrometric titration, and a continuous stirrer. Make the usual blank titration, using the glass electrode. Calculate results as directed in 60 (tentative, final action).

#### EDITORIAL RECOMMENDATION

(1) It is recommended that methods for the determination of phosphoric acid in basic slag be included with the other methods for phosphoric acid in fertilizers, *Methods of Analysis, A.O.A.C.*, 1940, instead of being separated by 14 pages. This can be done by making the following changes:

Delete 61. Change heading of 3 to read "Mechanical analysis of bone, tankage, and basic slag." Add to 3 the words: "With basic slag, use 10 g of material." Delete 62 and 63. Add to 8: "With basic slag proceed as directed in 8 (b)." Delete 64 and add to 9 the words: "With basic slag (then add remainder of 64)." Delete 65, and add this paragraph to 12 as (c), preceded by the words, "In basic slag." Place the material in 66, 67, 68 immediately after 17 with the heading, "Citric Acid-soluble Phosphoric Acid in Basic Slag—Official."

#### RECOMMENDATIONS FOR FURTHER WORK

- (1) That the study of the determination of moisture be continued.
- (2) That the study of the official ammonium citrate method for determining available  $P_2O_5$  in fertilizers be continued.
- (3) That work on the ferric sulfate and dipotassium phosphate method for nitrogen be discontinued and that the use of sodium phosphate of suitable pH be studied to ascertain whether it is better than magnesium oxide for ammoniacal nitrogen, 28, p. 27.
- (4) That methods for acid-soluble magnesium, magnesium activity, and acid-soluble manganese be further studied.
- (5) That work be continued on the method for acid- and base-forming qualities of fertilizer as recommended by the associate referee, including the method for eliminating basicity due to coarse material and the temperature of ignition.
- (6) That work on the details of the method for potash be continued, including the method for recovery of platinum.
- (7) That search for new methods for sulfur be continued and that the method for the determination of calcium be further studied.
- (8) That collaborative work be done on the volumetric method for total zinc in samples containing less than 0.1 per cent (*This Journal*, 26, p. 67), with the object of substituting it for the colorimetric method.

## NOTE

### Precipitation of Dextrin in Honey\*

The following experimental work was conducted with samples of honeydew honey containing 14.9 per cent of moisture and of buckwheat honey, containing 20.7 per cent of moisture (representing near-extremes in quantities of water involved), to determine the weight relationships between absolute alcohol, water, and sample charge existing in the mixture in which dextrin is precipitated according to the A.O.A.C. method for the determination of that constituent in honeys.

Charges of 4.0165 grams of the honeydew honey and 7.7293 grams of the buckwheat honey were weighed directly into tared, glass-stoppered, 100 ml. standard volumetric flasks; 4 ml. of distilled water was added to each charge, and the honeys were completely dissolved with slight warming. Absolute alcohol was added, a little at a time with vigorous mixing between additions, until each flask was filled to just under the mark. The stoppered flasks were then placed in the 20°C. room and left overnight. The next morning the volume was completed to exactly 100 ml. at 20°C., with absolute alcohol.†

After the tightly stoppered flasks had been allowed to come near enough to room temperature to obviate condensation of atmospheric moisture on their surfaces, they were weighed.

The following data were obtained:

	<i>With Honey- dew Honey</i>	<i>With Buck- wheat Honey</i>
Weight of honey (grams)	4.0165	7.7293
Water in honey (grams)	0.5985	1.6000
Water added (grams)	3.9830	3.9788
Water, total present (grams)	4.5815	5.5788
Abs. alcohol added (grams)	74.3426	72.3711
Vol. of abs. alcohol at 20° added (ml.)	94.183	91.686
Weight of total water + alcohol (grams)	78.9241	77.9499
Alcohol, by weight, in mixture of total water and alcohol (%)	94.195	92.84
Density of this alcoholic soln at 20°	0.8065	0.8103
Volume of this alcoholic soln at 20° (ml.)	97.86	96.20
Alcohol, by volume, at 20° (%)	96.24	95.30
Weight of honey dry-matter (grams)	3.4180	6.1293
Volume occupied by } ml. honey dry-matter } ml./g.	2.14 0.626	3.80 0.619
Weight of added water + alcohol (grams)	78.3256	76.3499
Alcohol, by weight, excluding total honey (%)	94.915	94.79
Density of this alcoholic soln at 20°	0.8045	0.8048
Volume of this alcoholic soln at 20°	97.362	94.865
Apparent volume occupied by } ml. charge of liquid honey } ml./g.	2.638 0.657	5.135 0.664

The results show that in the present A.O.A.C. method for determining dextrin in

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\* By George P. Walton, Special Commodities Branch, Office of Distribution, War Food Administration, Washington, D. C.

† 50 ml. of the same alcohol, at 20°C., weighed 39.445 grams. However, the weight of 0.78934 gram/ml., for absolute alcohol at 20°C., given in Bureau of Standards Circular No. 19, and also the density values given in Table 2 of the same circular were used in this study.

honey, the concentration of alcohol attained in the solution in which the dextrin is precipitated may vary from 95.30 to 96.24 per cent by volume, depending upon the weight of honey taken and its moisture content.

About the same proportionate variation occurs in the figures for apparent volume, in milliliters occupied per gram, by the charge of liquid honey. The actual variation, however, is only 0.007 ml. per gram, and the average value of 0.660 ml. per gram for the *apparent* volume occupied by the honey in the complete alcoholic mixture may be accepted as having general application in determining dextrin by the A.O.A.C. method. This suggests a means for calculating the quantity of absolute alcohol that must be added to complete the total volume of the mixture to 100 ml. (as required by the method), without actually carrying out the determination in a 100 ml. volumetric flask.

This procedure would permit modification of the method by weighing out the honey directly into a convenient type of flask (*e.g.*, a glass-stoppered Erlenmeyer), and controlling the requisite quantity of absolute alcohol to be added, either by weighing or by measuring the alcohol at 20°C.—an improvement in ease of operation and in precision.

To calculate the required quantity of absolute alcohol, a simple formula has been developed utilizing the data given in the table. Attention is directed especially to the figures for the weight of honey taken ("H") and the "per cent of alcohol by weight excluding total honey." This latter value varies only slightly in the two determinations, and the average value of 94.85 per cent alcohol by weight may be used for the general calculation without fear of introducing significant error. Necessarily, the quantity of water added as solvent for the honey must always be just 4 ml., as specified in the method.

Alcohol of 94.85 per cent by weight at 20°C. has a density of 0.80466 (g./ml.), and it is equivalent to 96.691 per cent alcohol by volume.

The other figure needed in the calculation is the average value of 0.66 ml. per gram, which represents the apparent volume occupied by the charge of honey and which was discussed previously.

Development of the formula follows:

Apparent volume occupied by honey = 0.66 H (ml.)

Volume of *added* water and alcohol = (100 - 0.66 H) (ml.)

But, as shown above, this added water and alcohol yield approximately 96.691 per cent alcohol, by volume, at 20°C.

Hence,

Volume of absolute alcohol at 20° to be added = .96691 (100 - 0.66H) ml.  
= 96.691 - 0.63816 H (ml.); or

Weight of absolute alcohol to be added = .78934 (96.691 - 0.63816H) g  
= 76.3221 - 0.50373 H (g.)

Note that if double quantities are being used throughout, and H is twice the weight that it would be if the total volume were to be 100 ml. instead of 200 ml., then the quantity of absolute alcohol to be used becomes 193.382 - 0.63816H (ml.), or 152.6442 - 0.50373H (grams), *not* twice the right-hand member of the respective equation.

After making a considerable number of calculations by the formulas and checking against various combinations of the tabulated data, the writer is convinced that they may be used to calculate the quantity of absolute alcohol to be added in precipitating dextrin by the A.O.A.C. method for its determination in honey without introducing significant error.

## BOOK REVIEWS

**A Source Book of Agricultural Chemistry.** By CHARLES A. BROWNE. Chronica Botanica Co., Waltham, Mass.; G. E. Stechert and Co., New York City. ix + 290 pages, 32 illustrations, subject and author indices, sup. roy. oct. Price, paper cover, \$5.00.

This work presents in biographic form and in chronological order the recorded literature of 2000 years of agricultural chemistry. Quite naturally the text treats not only of the literature of agricultural chemistry itself but also of chemistry in its broader aspects.

In a fine prefatory note the author states his purpose in undertaking the work and the reason for his choice of title. He points out that claims and statements of authors and eulogists (especially if not completely and critically read) are not always a reliable source of information. For example, he cites certain exaggerated claims that have been made by some of the overly ardent biographers of Liebig, the outstanding figure in agricultural chemistry; such statements, for example, as "that Liebig . . . was the first to point out the necessity of mineral matter in the nutriment of plants, that he was the first to indicate the interrelationship of plant and animal life, and that he was the first to show that the heat of the animal body is the result of processes of combustion performed within the organism." Careful inquiry shows that Liebig is not entitled to the full credit of such claims inasmuch as the statements quoted had long before been made by his predecessors. The quoted statement is but one of many grossly misleading assertions encountered by the author in his exhaustive readings of the scientific literature pertaining to agricultural chemistry, and the correction of such false information was one of the incentives that led to the preparation of the present volume.

An attractive feature of the present book is indicated in the following quotations from the preface: "[The author] has preferred so far as possible to let these predecessors in selected passages give their own accounts of the work selected for description, with no attempts at modernization of language." And again: "The author has attempted to give a balanced presentation of the stories of truth and error as they have developed side by side." The reviewer feels that the record has been set straight, once and for all, and that the author has indeed given "a more accurate and complete account than has hitherto appeared, of the origins of agricultural chemistry."

Dr. Browne is to be commended upon the thorough and erudite manner in which he has handled his subject. Step by step he unfolds the fascinating story of the progress of agricultural chemical research, beginning with the Greek philosopher Thales (about 640 B.C.) and ending with Justus von Liebig and a review of his numerous works, including his immortal book "*Organische Chemie in Ihrer Anwendung auf Agrikultur und Physiologie*," which was published in 1840.

Much of the delight in reading this history comes from the wise decision of the author to let the 40 or more authors presented speak their own lines. These are woven together into a clear and delightful pattern by the author's interspersed comment on the scientific importance of the various discoveries and advances in the light of the present day knowledge of agricultural chemistry.

The reviewer cannot repress the desire to pay tribute to the keen sense of observation and inductive reasoning that these early investigators possessed. An outstanding example is the clear conception of the nature of matter as announced by Democritus almost two thousand years before Dalton. This philosopher viewed matter as consisting of exceedingly small indivisible particles. He also conceived the idea of the indestructibility of matter, and that changes of matter are the result of disintegration in which older atomic combinations are transformed into new arrange-



ments. As Dr. Browne so aptly says: "The atomic conception of Democritus is one of the greatest achievements of the human mind and had it met with immediate acceptance the rise of modern chemistry might have been advanced two thousand years."

The book contains many fascinating cuts of original laboratory apparatus and reproductions of title pages of some of the outstanding publications, as well as a cut of Redi's original table (1698) of his early determinations of the ash content of plants.

Except for a few minor slips, such as "tabuates" for tabulates (p. 79), "Frankfurt am Oder" instead of Frankfurt an der Oder (p. 116), and the accidental upsetting of a line on page 266, the typographical appearance of the volume is excellent. Bibliographies at the end of each section of the book and an addendum of works dealing with the later history of agricultural chemistry in the century 1840-1940, supply useful references to original source material.

The reviewer feels no hesitancy in recommending this work to the entire chemical profession, not only because of its solid historical value, but, too, because of the thorough and vivid manner in which it portrays the splendid scientific achievements of the pioneers of agricultural chemistry. The book fills a decided need, and the hope is expressed that the author in a later volume may extend his history of agricultural chemistry to the post-Liebig period.—B. G. HARTMANN.

**Organic Chemistry.** By LOUIS F. FIESER, Professor, Converse Memorial Laboratory, Harvard University, and MARY FIESER, his wife. D. C. Heath and Co., Boston, Mass. 1944. XII + 1091 pp. Price, \$8.00. College Edition, \$6.00.

In slightly more than 1000 pages the authors have presented a well-written, interesting, and comprehensive picture of the field of organic chemistry. To do that in this limited space, it was necessary to concentrate on the most important steps in the development of the subject matter, and with few exceptions this objective has been accomplished. For the reader who wishes further details there are given, at the end of each chapter, references to up-to-date publications, where the particular subject has been treated more exhaustively. A few subjects of special interest have been dealt with more fully than the general pattern of the book would require, but this plan has added to rather than detracted from the value of the book, since it has been done in the separate chapters that appear at intervals throughout the book. Those interested in the volume only as a textbook may ignore these chapters without interrupting the continuity of the text, but those who wish a summary of up-to-the-minute developments in fields other than their own will find these chapters welcome nuggets of condensed information, as pleasing to encounter as nuts in a candy bar. Thus the reader, in a few minutes, may gain an insight into the latest developments on synthetic rubber, sulfa drugs, the vitamins, or any of the several other subjects treated in these special chapters. Because of the space taken up by these chapters in the trade edition, the college edition, while covering the same general subject matter is, we understand, abridged somewhat to give a smaller volume.

A timely chapter on synthetic rubber presents a welcome vignette of a topic of keen personal interest to almost every present day reader. The chapter on dyestuffs is also well done in the presentation of a subject on which too little of a reliable nature has been written.

In the treatment of the fundamental classes of organic compounds, biochemistry is stressed. This is as it should be, since this subject comprises a large field in organic chemistry. The part that important compounds play as by-products of, or participants in, the metabolic processes of living organisms is revealed and emphasized, and the effect produced increases the reader's interest by lending reality and practical

importance to the compounds so discussed. Fundamental and characteristic reactions are given for each class of compounds, both those by which the compound may be synthesized and those into which it enters, and the part that each class plays in reactions of the Friedel and Craft type and of the Grignard procedure is particularly emphasized. The yields, kinds of catalysts, and other conditions of each reaction are also set forth in most cases. Where pertinent, interesting bits of historical background have been added in a paragraph or two, which sometimes give a revealing glimpse of the influence which economic and political forces have exerted on the evolution of the science of organic chemistry, and they also often impress one with the caliber of the men who have advanced our knowledge in this field, and their capacity for sound, incisive thinking.

Adverse comment might justly be directed at the somewhat undue amount of space given to speculation over the mechanics of certain reactions. When such speculation invokes the invention of a plausible intermediate compound to bridge the gap between starting material and end product it seems unwarranted in a book of this scope to give more than a passing comment; otherwise the discussion tends to become abstract and the reader's interest wanes.

The authors have used several devices, such as new prefixes, which while effective add more variants to an already complex nomenclature, in which every effort should be made for conformity and uniformity.

The errors of composition are few and unimportant, and they do not detract from the value of the book, which on the whole is an accurate, concisely composed exposition of our present knowledge of organic chemistry. The book should prove a valuable addition to the organic chemist's library.—L. M. BEACHAM.

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TABLE 1.—*Collaborative results*  
(Percentages)

CHEM.	VACUUM DRIED 100° C			AIR DRIED 100° C			VACUUM DRIED 70° C			AIR DRIED 70° C			TOLUENE
	ACID.	SOLIDS	ACID.	SOLIDS	ACID.	SOLIDS	SOLIDS	ACID.	SOLIDS	SOLIDS	ACID.	SOLIDS	SOLIDS
	A	B(1)	B(2)	B(3)	C(1)	C(2)	C(3)	D(1)	D(2)	D(3)	E(1)	E(2)	E(3)
1	5.45	27.8	5.14	29.2	27.6	6.79*	28.0	29.8	7.21*	28.6	29.9	6.08*	29.9
2	5.51	28.72	3.61	30.38	27.54	2.64	28.75	30.03	4.48	31.49	30.31	4.32	31.09
3	5.44	26.97	3.98	28.21	27.42	4.82	28.98	28.91	5.04	29.31	29.22	5.41	29.29
4 a.		28.39	4.07		28.26	3.51							29.99
5 b.	5.3	26.22	4.4	28.51	25.06	1.6*	26.65	29.39	4.7	28.85	29.28	4.3	29.66
6	5.68	28.6	4.22	28.9	27.8	5.17	28.3	29.9	5.40	29.0	28.8	4.61	27.8
7	5.45	27.43	3.92	28.25	25.45	2.21	27.04	29.48	4.90	28.60	28.72	5.00	28.43
8	5.22	27.46	4.44	28.00	27.23	4.59	27.90	30.43	4.49	28.83	29.62	4.78	28.50
9	5.61	27.82	4.59	27.90	28.61	3.10	28.67	28.77	5.39	28.83	29.56	5.30	29.09
10 b.	5.49				27.17	4.26					27.94	4.74	
Ave.	5.46	27.71	4.26	28.67	27.21	3.79	28.04	29.59	4.91	29.19	29.26	4.81	29.22
													29.98

\* Omitted from average.

a. Average of 3 tests.

b. Average of 2 tests.

upon which condensed buttermilk is sold, it was decided to begin at this point in attacking the problem.

Except for the already established methods of the A.O.A.C. for solids determination on milk products, very little information was to be had from a literature search. However, two points were established: (1) On drying of buttermilk products, a charring of the sample is noted; (2) Lactic acid of buttermilk products decomposes when heated. A note by J. W. E. Harrison appearing in *This Journal*, 18, 645 (1935) recommends the use of an agent such as zinc oxide to prevent loss of lactic acid and any unfavorable action it might have on milk solids when drying.

Consequently, collaborators, who had shown their willingness to co-operate in a gratifying manner, were sent a semi-solid buttermilk sample with following instructions:

- (A) Determine acidity of sample.
- (B) Determine solids at 100°C. under vacuum. (A.O.A.C. method). Determine acidity of dried sample.
- (C) Determine solids at 100°C. without vacuum. Determine acidity of dried sample.
- (D) Determine solids at 70°C. with vacuum. Determine acidity of dried sample.
- (E) Determine solids at 70°C. without vacuum. Determine acidity of dried sample.
- (F) Determine solids by toluene distillation method.

The collaborators were further asked to repeat the above determinations, this time adding 2 grams of recently ignited zinc oxide, 5 ml of water, and mixing thoroughly. Results are shown in Table 1 under B(3), C(3), D(3), and E(3).

List of collaborators:

- (1) Wm. L. Hunter and Van P. Entevistle, California
- (2) Harry J. Fisher and Owen L. Nolan, Connecticut
- (3) P. B. Curtis and A. E. Rihn, Indiana
- (4) L. E. Bopst and H. R. Walls, Maryland
- (5) Rodney C. Berry and Jas. A. Johnson, Virginia
- (6) W. C. Geagley and Percy O'Meara, Michigan
- (7) Howard Hammond, North Dakota
- (8) Guy G. Frary and E. H. Zilliox, South Dakota
- (9) J. F. Fudge and T. L. Ogier, Texas
- (10) L. S. Walker, Vermont
- (11) J. W. Kuzmeski and C. Tyson Smith, Massachusetts

#### COMMENTS OF COLLABORATORS

- (1) Samples on drying darkened from light brown to black. Zinc oxide plus water addition shortened time to come to constant weight. Spattering noted when applying vacuum.
- (3) Residue very dark and difficult to dissolve after drying at 100°C., but the ZnO residue is white. Residue is unchanged after drying at 70°C.
- (5) In titration of charred samples end point is difficult to determine. 14 to 31 hours needed for drying at 70°C.

- (8) ZnO samples spattered. Thirty minutes on steam bath before placing in oven prevented this. ZnO seemed to prevent charring.
- (9) Residues brittle and almost black; difficult to read end point.
- (10) Trouble reading indicator end point. Required 22 hours to reach constant weight at 70°C.
- (11) It seems impractical to dry the type of material submitted to constant weight in the usually accepted sense of the term by any of the methods used to obtain the results reported.

Results and comments of the collaborators verify sample charring and lactic acid loss on drying of buttermilk products. However, this is eliminated somewhat at the lower temperature. Length of time required would be a definite disadvantage to the use of the lower temperature (70°C). The use of zinc oxide seems to aid the situation, indicating that the neutralization of the lactic acid tends to prevent the charring. Use of vacuum minimizes the lactic acid loss, as does also the use of the lower drying temperature. Three collaborators made the acidity test on the zinc oxide dried samples and their results, shown in Table 2, indicate that the lactic acid was not neutralized by the zinc oxide.

TABLE 2.—*Per cent lactic acid in ZnO dried samples*

CHEMIST	a(4)	c(4)	d(4)	e(4)
8	3.80	3.59	2.59	2.31
5	4.20	4.50	3.90	2.90
9	3.57	3.60	3.16	3.57
Ave.	3.86	3.87	3.22	2.93

If the loss in lactic acid is added to the total solids content in the vacuum and air dried samples the results in the case of 100°C. drying would be 28.91 per cent and 28.88 per cent, respectively; while in the case of 70°C. drying they would be 30.14 per cent and 29.91 per cent.

Considering the type of sample used the results of the solids content determinations are in good agreement. However, because of the charring, indicating an oxidation possibility, and the incomplete neutralization of the lactic acid in the zinc oxide experiment, further work on this problem is contemplated.

The sampling procedure now most frequently used is shaking of barrel or container, or the use of a wooden paddle in agitating the product and then taking a sample when the material is believed to be uniform. Another method is to use a special tool which draws a core, about three-fourths of an inch in diameter, from top to bottom of container. This tool is constructed especially for liquids and semisolid products. Samples drawn by these methods will be used in further collaborative work.

It is recommended\* that collaborative work on the sampling and analysis of condensed buttermilk be continued.

\* For report of Subcommittee A and action of the Association, see *This Journal*, 30, 41 (1947).

## CONTRIBUTED PAPERS

### THE DETERMINATION OF MOISTURE IN FERTILIZERS<sup>1</sup>

By WILLIAM H. ROSS<sup>2</sup> and KATHARINE S. LOVE (Division of Fertilizer and Agricultural Lime, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland)

The present official method for the determination of moisture in fertilizers was adopted by this Association at its second annual meeting in 1885 (4). The method consists in drying the sample at a temperature of 98°–100°C. for a period of 5 hours. The loss in weight includes free moisture, occasional volatile decomposition products from constituents of the sample, and usually more or less water of crystallization from monocalcium phosphate and other hydrated constituents. Inasmuch as all superphosphates and mixed fertilizers of the ordinary type contain some water of crystallization or hydration, and since many mixed fertilizers also contain easily decomposable materials, it must follow that this method cannot be depended upon to give accurate results for free moisture in all types of materials and mixtures.

At the 1931 meeting of the Association a second procedure was recommended for adoption as a tentative method (6) for the determination of moisture in fertilizers. This method consists in subjecting the sample to distillation with toluene for a period of 1–10 hours depending on the nature of the material being analyzed. The boiling point of toluene is 110.8°C. This temperature is still higher than that recommended by the official method and thus this method is also capable of liberating volatile reaction products and water of hydration from the sample. This tentative method is, therefore, likewise unsuited for the determination of free moisture in mixed fertilizers and many fertilizer materials.

A recommendation was accordingly adopted at the 1940 meeting of this Association that the Associate Referee on phosphoric acid make a study of the methods for the determination of moisture in fertilizers. As a preliminary to this study, Whittaker and Ross (7) in 1941 reviewed the various methods that were then in use or had been proposed for the determination of moisture. The view was expressed that the problem of determining free moisture in fertilizers could be solved by drawing heated air through the sample at a constant temperature of 60°C. At this temperature there is little or no decomposition of hydrated salts that commonly occur in mixed fertilizers and the decomposition or oxidation reactions that cause so much trouble in present methods are reduced. The equipment designed for this rapid removal of moisture at a relatively low temperature is de-

<sup>1</sup> Presented at the annual meeting of the Association of Official Agricultural Chemists, at the Thursday morning session, October 26, 1944.

<sup>2</sup> Deceased.

TABLE 1.—*Ammonium nitrate samples*

SAMPLE NO.	SOURCE	TYPE OF GRANULES	TREATMENT	MOISTURE IN SAMPLE
ANPL #1	Alberta Nitrogen Products, Ltd.	Sprayed	None	Original
ANPL #2	" "	"	0.5% WP <sup>1</sup> +3.5% Kittitas	Added
WCW #3	Welland Chemical Works	"	None	Original
TVA #4	Tennessee Valley Authority	Grained	"	Absorbed
TVA #5	" "	"	1% PRP <sup>2</sup> +4% Celite	"
MCW #6	Military Chemical Works	Crystallized	None	Original
MCW #7	" "	"	"	"

<sup>1</sup> Paraffin-petrolatum coating.<sup>2</sup> Petrolatum-resin-paraffin coating

scribed by Hardesty, Whittaker, and Ross elsewhere in this issue under the title of "The Air-flow Method for the Determination of Moisture in Fertilizers." (2).

During the past few years solid ammonium nitrate has become an important source of fertilizer nitrogen. Since small amounts of moisture have a profound effect on its physical condition, an accurate method of determining its moisture content is essential to the improvement and control of the mechanical condition of this material. Ammonium nitrate undergoes slow decomposition at 100°C. and it is desirable to know the adaptability of the official methods and other procedures for determining its moisture content.

With the view to finding a suitable method for this determination a set of ammonium nitrate samples was prepared and submitted to a number of laboratories accustomed to making moisture determinations in this material. The samples were accompanied with the request that they be analyzed for moisture by such methods as were considered to be best suited for ammonium nitrate.

The samples prepared for this collaborative study are given in Table 1. Sample ANPL #1 was selected from a commercial shipment that had been stored in a dry place. Sample ANPL #2 was also selected from a commercial shipment but its moisture content was increased by spraying it with water while being rolled in a rotating drum. Samples TVA #4 and TVA #5 were taken from commercial shipments that had been stored in bags for a period of three months at 55°F. and a relative humidity of 80 per cent. Sample WCW #3 was collected at the plant before it was dried to the point desired for shipment. Samples MCW #6 and MCW #7 were prepared on a pilot plant scale. The former was dried as completely as was considered practical under commercial conditions. Sample MCW #7 was represented as being only partially dried. The moisture in Samples ANPL #1 and ANPL #2 and in Samples TVA #4 and TVA #5 includes that which had been taken up by the samples subsequent to thorough drying at the factory. The moisture in Sample WCW #3 and in Samples MCW #6 and #7, on the other hand, represents original moisture that had not been eliminated by drying.

The methods used by the collaborators for the determination of moisture in the samples and the results obtained are shown in Table 2. The results as a whole show good agreement considering the hygroscopic nature of the samples submitted for analysis. Good results were obtained with the Fischer method which consists of solvent-extraction of moisture from the sample and determination of the amount of moisture in the solvent by means of Karl Fischer Reagent (1, 5). This method is rapid and seems to be quite well adapted for the determination of moisture in ammonium nitrate. It, however, gives water of crystallization as well as free moisture when both are present and it is, therefore, not suited for the

TABLE 2.—*Moisture in ammonium nitrate samples*  
Percentages

COLLABORATOR	METHODS	ANAL.						
		#1	#2	#3	#4	#5	#6	#7
Alberta Nitrogen Products, Ltd. Hercules Powder Company U. S. Department of Agriculture Consolidated Mining and Smelting Company Military Chemical Works Military Chemical Works Eastern States Farmers' Exchange Tennessee Valley Authority	Oven-drying	0.13	2.97	0.74	0.75	0.92	0.17	0.75
	2 hrs. at 90°C.	0.11	2.70	0.50	0.57	0.85	0.11	0.44
	2 hrs. at 95°C.	0.08	2.91	0.74	0.75	0.78	0.08	0.66
	20 hrs. at 70°C.	0.08	2.75	0.72	0.65	0.80	0.08	0.55
	4 hrs. at 80°-85°C.	0.06	2.59	0.64	0.47	0.71	0.11	0.47
	5 hrs. at 70°C.	0.08	2.76	0.71	0.56	0.88	0.09	0.42
	2 hrs. at 100°C.	0.11	2.79	0.76	0.61	0.81	0.09	0.43
	5 hrs. at 100°C.	0.09	2.84	0.82	0.71	0.83	0.09	0.73
	20 hrs. at 70°C.							
	Air-flow							
U. S. Department of Agriculture Welland Chemical Works Eastern States Farmers' Exchange Tennessee Valley Authority Tennessee Valley Authority	Air at 70°C. for 4 hrs.	0.07	2.89	0.80	0.79	0.80	0.08	0.60
	Dry air at 65°-70°C. for 40 minutes	0.07	2.69	0.78	0.61	0.70	0.06	0.33
	Air at 60°C. for 3 hrs.	0.09	2.73	0.78	0.58	0.77	0.09	0.47
	Dry air at 60°C. for 6 hrs.	0.07	2.72	0.75	0.78	0.81	0.08	0.67
	Dry air at 70°C. for 5 hrs.	0.08	2.68	0.91	0.83	0.79	0.10	0.60
	Other Procedures							
	2 hrs. in vacuum at 80°C.	0.09	2.67	0.89	0.68	0.83	0.07	0.71
	Fischer	0.09	2.52	0.72	0.70	0.99	0.09	0.85
	Mean	0.09	2.75	0.75	0.67	0.82	0.09	0.58
Solvay Process Company								
Alberta Nitrogen Products, Ltd.								

determination of free moisture in superphosphate and mixed fertilizers. The other methods used by the collaborators included ordinary oven drying at temperatures of 100°C. or below, and drying by the air-flow method for varying lengths of time and at different temperatures.

The rate at which ammonium nitrate undergoes drying by these two methods and by the method of drying at room temperature in a vacuum over "Anhydrone" is shown by the data in Table 3. The results in the table indicate that drawing air at 70°C. through the sample for 3 hours by air-flow method is equivalent to 18–24 hours drying in a static air oven at

TABLE 3.—Rates at which samples of ammonium nitrate decrease in weight on drying by different methods

TIME OF DRYING, HOURS	PER CENT LOSS IN WEIGHT OF SAMPLE						
	ANPL		WCW	TVA		MCW	
	#1	#2	#3	#4	#5	#6	#7
In Air Oven at 70°C.							
6	0.07	2.83	0.66	0.66	0.71	0.05	0.48
12	0.08	2.88	0.72	0.73	0.76	0.08	0.59
18	0.08	2.90	0.74	0.75	0.78	0.08	0.65
24	0.05	2.88	0.74	0.75	0.77	0.06	0.67
30	0.08	2.92	0.76	0.78	0.80	0.09	0.68
In Vacuum over Anhydrone at Room Temperature							
6	0.00	2.79	0.36	0.65	0.70	0.00	0.28
12	0.00	2.80	0.36	0.69	0.74	0.00	0.28
18	0.01	2.82	0.38	0.70	0.75	0.01	0.28
24	0.01	2.85	0.40	0.73	0.79	0.02	0.29
30	0.01	2.85	0.39	0.73	0.79	0.02	0.29
Air-flow at 70°C.							
1		2.82	0.58	0.73	0.68	0.03	0.41
2		2.87	0.72	0.77	0.74	0.07	0.57
3		2.90	0.82	0.81	0.79	0.09	0.71
4		2.90	0.85	0.81	0.79	0.10	0.75
5		2.93	0.89	0.83	0.85	0.12	0.78

the same temperature. The air-flow method thus has the advantage of being more rapid than the method of drying in an air oven and it is, therefore, better adapted for use in control work. The table further shows that samples, such as ANPL #2, TVA #4 and TVA #5, which contained only added or absorbed moisture, can be dried in a vacuum over Anhydrone at room temperature, but that this method of drying does not eliminate within a period of 30 hours all the original or occluded moisture in such samples as WCW #3 and MCW #7.

\* Commercial name for anhydrous magnesium perchlorate.



## OTHER FERTILIZER MATERIALS

The results obtained in a comparison of the official method, the vacuum-drying method and the air-flow method for the determination of moisture in other fertilizer materials are shown in Table 4. The three methods give essentially the same results for the stable materials, ammonium sulfate and potassium chloride. The higher values obtained by the official method on the other materials listed in Table 4 are attributable to a number of factors (a) decomposition of the compound such as may occur slowly with ammonium nitrate at 100°C., (b) loss of water of crystallization such as

TABLE 4.—*Moisture in fertilizer materials as determined by different methods*  
Percentages

TIME OF DRYING	MONO- CALCIUM PHOSPHATE MONO- HYDRATE <sup>1</sup>	MONO- CALCIUM PHOSPHATE MONO- HYDRATE <sup>2</sup>	DICALCIUM PHOSPHATE DIHYDRATE	SUPER- PHOSPHATE	DOUBLE SUPER- PHOSPHATE	AMMONIUM SULFATE	POTASSIUM CHLORIDE	AMMONIUM NITRATE
Official Method								
3	0.10	0.95	2.99	8.55	4.03	—	0.05	0.17
5	0.09	1.15	16.16	8.61	4.05	1.78	0.05	0.30
7	0.13	1.27	17.95	8.66	4.18	—	0.06	0.40
In Vacuum over Anhydronc at Room Temperature								
16	0.00	0.56	0.00	8.30	3.08	—	0.00	0.00
21	0.00	0.58	0.00	8.33	3.09	1.80	0.00	0.00
26	0.00	0.58	0.32	8.41	3.14	—	0.00	0.00
Air-flow Method at 60°C.								
1	0.03	0.32	0.02	7.25	2.60	—	0.01	0.14
2	0.02	0.33	0.02	7.42	2.76	1.72	0.00	0.23
3	0.03	0.33	0.04	7.39	2.75	—	0.01	0.32

<sup>1</sup> Washed with acetone.

<sup>2</sup> Contains slight amount of free acid.

may occur at 100°C. with C. P. monocalcium phosphate, dicalcium phosphate, or the superphosphates used in these tests, and (c) the vapor pressure of the liquid phase of monocalcium phosphate and the superphosphates containing free phosphoric acid is higher at 100°C. than at lower temperatures, which may account in part for the higher values obtained at 100°C.

The results in Table 4 also indicate that drying in vacuum over Anhydronc does not remove occluded moisture from ammonium nitrate. On the other hand, it gives higher results for moisture in dicalcium phosphate dihydrate after 26 hours in the desiccator than those obtained by the air-flow method. As compared with the air-flow method it also has a tendency to give considerably higher results for materials containing free phosphoric

acid, namely, the unwashed monocalcium phosphate monohydrate and the superphosphates used in these tests.

### CONCLUSIONS

Under the conditions of these tests the following conclusions would seem valid for the materials and methods studied.

1. The official method usually gives higher results for moisture in fertilizer materials containing water of hydration than either of the other two methods.

2. The air-flow method gives lower results for moisture in materials that tend to lose water of hydration in vacuum over Anhydrone than either the official or the vacuum-drying methods.

3. The presence of free acid, as observed by Hill and Jacob (3), increases the rate at which monocalcium phosphate monohydrate loses water of hydration when dried as directed in the official method.

4. The air-flow method gives higher results than the vacuum-drying method for moisture in a material such as ammonium nitrate which contains occluded water.

5. Although ammonium nitrate is known to undergo decomposition at 100°C, the rate of decomposition is too slow to seriously interfere with the determination of moisture in this material by the official method.

6. All three methods give essentially the same results for moisture in stable materials that do not contain occluded water or water of crystallization.

7. One or two hours' drying by the air-flow method at 60°C. would seem to suffice for the determination of moisture in all samples used in the tests with the possible exception of crystalline ammonium nitrate containing occluded water.

8. The official method is not adapted to the determination of free moisture in the presence of easily oxidizable organic materials, as observed by Hardesty, Whittaker, and Ross in the accompanying paper (2), since a temperature of 100°C. may cause decomposition and loss in weight of the sample other than that representing the free-moisture content.

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FACTORS AFFECTING THE AVAILABILITY OF  
AMMONIATED SUPERPHOSPHATES. PART II.THE EFFICIENCY OF AMMONIATED SUPERPHOSPHATES AS  
INDICATED BY POT TESTS IN THE GREENHOUSE<sup>1</sup>By WILLIAM H. ROSS,<sup>2</sup> J. RICHARD ADAMS,<sup>3</sup> JOHN O. HARDESTY,  
AND COLIN W. WHITTAKER<sup>4</sup>

Superphosphates that have been ammoniated differ from those of the ordinary type in that they contain a higher proportion of phosphatic components soluble in neutral ammonium citrate solution. Inasmuch as the availability of a phosphatic material as determined by the official method depends on its solubility in neutral ammonium citrate solution, it would be expected, and has actually been found by analysis, that the method indicates a higher availability for the  $P_2O_5$  in a mixture containing a low proportion of an ammoniated superphosphate than in one containing a high proportion of the same material. This failure to indicate a constant availability for a given material is a serious defect in the official method. In compliance with a recommendation that was adopted by the Association in 1942, a further study was undertaken of (a) the factors that affect the chemical availability of the  $P_2O_5$  in ammoniated mixtures; and (b) the manner in which the same factors affect the efficiency of an ammoniated superphosphate in promoting crop growth.

The results of the study of the factors affecting the chemical availability of ammoniated mixtures, namely: (a) degree of ammoniation, (b) storage temperature following ammoniation, (c) moisture content, (d) presence of dolomite, and (e) presence of fluorides, were presented in Part I of this report (2). The present paper presents results of a collaborative study dealing with the effects of the foregoing factors on the efficiency of ammoniated mixtures in promoting crop growth in the greenhouse, and is a continuation of the work recommended by the Association in 1942.

## PROCEDURE

The fertilizer used in these tests consisted of 5-6-8 grade prepared from 4-12-4 ammoniated base after 36 days storage of the base at temperatures of 20, 60, and 90°C. The dolomite, when present during ammoniation and storage, was added at the rate of 250 pounds per ton of the 4-12-4 base mixture prior to ammoniation. All of the 5-6-8 mixtures and the 5-0-8 check mixture, as applied to the soil, contained 303 pounds of dolomite per ton regardless of the dolomite status of the 4-12-4 base mixture. There-

<sup>1</sup> Contribution from the Division of Fertilizer, and Agricultural Lime Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Md.

<sup>2</sup> Deceased.

<sup>3</sup> Present address, Spencer Chemical Company, Kansas City, Mo.

<sup>4</sup> In collaboration with W. B. Andrews, Mississippi Agr. Experiment Station, R. P. Bartholomew, Arkansas Agr. Experiment Station, W. R. Paden, South Carolina Agr. Experiment Station, G. W. Volk, Alabama Polytechnic Institute.

fore, in this paper, the references to the presence or absence of dolomite refer to the 4-12-4 base mixture during ammoniation and storage. The relationships of the results reported might be quite different if dolomite were not present in all of the 5-6-8 mixtures. The dolomite used had a fineness of 90% through a standard 100 mesh screen. The aqua ammonia added was varied to give ammoniated mixtures containing 9 per cent moisture and 0, 2, 3, 4, and 5 parts of ammonia per 100 parts of ammoniated superphosphate on the basis of 20 per cent available  $P_2O_5$  in the superphosphate used. Anhydrous ammonia was used to ammoniate mixtures that were formulated to contain only 3 per cent moisture.

Nitrogen in the completed mixtures was supplied mainly as ammonium sulfate and sodium nitrate, the same amounts of these materials being used in all cases. Because, however, of the variable amount of nitrogen in the form of ammoniated superphosphate it was necessary to adjust the total amount of nitrogen in order to have the same percentages of nitrogen present in all mixtures. This was done by adding the required amounts of urea. Potash was supplied as high grade potassium chloride.

The test crops included millet, wheat, Sudan grass, and sorghum. The fertilizer was applied to the millet and wheat crops by mixing the required amount with 5 per cent of the soil in the pot and depositing this mixture in a horizontal layer halfway between the bottom and top of the pot. With the Sudan grass and sorghum the fertilizer was located in a circular area about 3 inches in diameter and 1.5 inches from the top of the soil. The seed were planted in a ring 5 inches in diameter concentric with the fertilized area. Both methods of placement were used with sorghum on the Atwood fine sandy loam.

Four different brands of superphosphate were compared in the tests. Three (A, B, and C) were made from Florida pebble and one (D) from Tennessee brown rock phosphate. Superphosphate A was used in most of the tests. The average yields obtained in the pot tests (with isolated exceptions) indicated no significant difference, either before or after ammoniation, in the availability to plants of the  $P_2O_5$  in these superphosphates from different sources. Other superphosphates used in the tests of the effect of fluorine will be described later.

Seven different acid soils (pH range 4.6 to 5.9) at 5 locations, and an alkaline soil, the Houston clay (pH 8.2) at one of the locations, were used in the study. The conditions under which the tests were made with the different soils and crops, and the mean yields obtained in the non-ammoniated and no-phosphorus check pots, are given in Table 1. These phosphorus deficient soils were chosen to afford accurate measurement of small differences in the efficiency of the phosphates. The relative efficiency of applied phosphates may be affected by the level of available phosphorus in the soil. If that be the case, the data secured on these soils may

TABLE 1.—Conditions of pot experiments

COOPERATING ORGANIZATION	SOIL			P <sub>2</sub> O <sub>5</sub> RATE PER ACRE	CROP			AV. YIELD DRY WT. PER POT		REFRACTIONS
	TYPE	pH	AIR-DRY WT. PER POT		KIND	GROWING PERIOD	PLANTS PER POT	WITH P <sub>2</sub> O <sub>5</sub>	WITHOUT P <sub>2</sub> O <sub>5</sub>	
								grams	grams	
Bur. Plant Industry	Hartsells fine sandy loam	5.5	lb. 11.2	lb. 80	Millet	days 45	5	11.7	0.7	5
"	Wooster silt loam	4.9	11.6	80	Millet	62	5	36.1	14.4	5
"	"	4.9	11.5	40	Wheat	137	5	31.3	9.3	3
"	Sassafras sandy loam	4.6	11.2	70	Wheat	124	5	32.3	0.7	3
Arkansas Experiment Station	Newtonia silt loam	5.9	15.0	120	Sudan grass	49 <sup>2</sup>	16	25.9	3.9	6
Alabama Experiment Station	Cecil clay	5.7	—	—	Sudan grass	—	—	23.7	0.9	3
South Carolina Experiment Station	Grady sandy loam	5.5	—	—	Sudan grass	95	—	24.3	10.8	2
Mississippi Experiment Station	{ Atwood fine sandy loam Houston clay	5.9	24.0	60	Sorghum	84	8	35.2	15.7	4
		8.2	15.0	60	Sorghum	58	8	21.7	15.7	4

<sup>1</sup> Non-ammoniated.<sup>2</sup> First cutting.

not be readily interpretable in terms of soils higher in available phosphorus.

Average yields and differences required for significance are given in the Appendix, Sections 1 and 2. To facilitate discussion the data have been computed to relative yield increases and are used in that form in the tables in the body of the paper.

#### DEGREE OF AMMONIATION

The effect of degree of ammoniation on the availability to plants of the  $P_2O_5$  in ammoniated superphosphate mixtures is indicated by the results in Table 2. This table shows the average relative increase in yield of millet, wheat, Sudan grass, and sorghum when fertilized with superphosphate mixtures that have been ammoniated to 0, 2, 3, 4, and 5 per cent on

TABLE 2.—*Effect of degree of ammoniation on the availability to plants of the  $P_2O_5$  in ammoniated superphosphate mixtures. Dolomite present; moisture 9 per cent; temperature of storage 20°C.*

TEST MIXTURE	DEGREE OF AMMONIATION: NH <sub>3</sub> ADDED ON BASIS OF SUPERPHOSPHATE IN MIXTURE	AVERAGE RELATIVE INCREASE IN YIELD <sup>1</sup>
No.	Per cent	Per cent
1	0	100
2	2	100
3	3	88
4	4	82
5	5	65

<sup>1</sup> On the basis of 100 as the average relative increase for the non-ammoniated mixture.

the basis of the superphosphate in the mixture. The results show that a 2 per cent ammoniated superphosphate mixture is as available to plants as a non-ammoniated mixture but that the efficiency of the ammoniated superphosphate decreases as the degree of ammoniation is increased to and beyond 3 per cent.

#### TEMPERATURE OF STORAGE

The samples that were ammoniated from 2 to 5 per cent were stored at temperatures of 20°, 60°, and 90°C. for 36 days. The results in Table 3 show the average relative increases in yield obtained in pot tests with the mixtures stored at different temperatures. The data in the table are consistent in showing that the availability to plants of the  $P_2O_5$  in mixtures containing dolomite decreases, not only with increase in the rate of ammoniation but also with increase in the temperature at which the samples are stored following ammoniation. The data further show that the effect of temperature in the range 60°–90°C. is more pronounced for the lightly than for the heavily ammoniated mixtures.

TABLE 3.—*Effect of temperature of storage on the availability to plants of the  $P_2O_5$  in ammoniated superphosphate mixtures, dolomite present; moisture 9 per cent*

TEST MIXTURE	DEGREE OF AMMONIATION: $NH_3$ ADDED ON BASIS OF SUPERPHOSPHATE IN MIXTURE	STORAGE TEMPERATURE	AVERAGE RELATIVE INCREASE <sup>1</sup>
No.	Per cent	°C.	Per cent
2	2	20	100
3	3	20	88
4	4	20	82
5	5	20	65
6	2	60	93
7	3	60	68
8	4	60	62
9	5	60	55
10	2	90	76
11	3	90	59
12	4	90	53
13	5	90	53

<sup>1</sup> On the basis of 100 as the average relative increase for the non-ammoniated mixture.

## EFFECT OF MOISTURE

The effect of the moisture content of an ammoniated superphosphate mixture on the availability of its  $P_2O_5$  to plants is shown by the data in Table 4. The samples used in this series of tests were all ammoniated to 5 per cent on the basis of the superphosphate in the sample. The average results reported by all the collaborators were consistent in showing that the  $P_2O_5$  in samples ammoniated at a moisture content of only 3 per cent is somewhat more available to plants than that in samples that were am-

TABLE 4.—*Effect of moisture on the availability to plants of the  $P_2O_5$  in ammoniated superphosphate mixtures. Degree of ammoniation, 5 per cent; dolomite present*

TEST MIXTURE	MOISTURE	TEMPERATURE OF STORAGE	AVERAGE RELATIVE INCREASE IN YIELD <sup>1</sup>
No.	Per cent	°C.	Per cent
14	3	20	78
5	9	20	65
15	3	60	66
9	9	60	55
16	3	90	75
13	9	90	53

<sup>1</sup> On the basis of 100 as the average relative increase for the non-ammoniated mixture.

moniated at a moisture content of 9 per cent, and that the effect of the moisture was most pronounced at the highest temperature, 90°C.

## EFFECT OF DOLOMITE

The effect of the presence of dolomite during ammoniation and storage on the availability to plants of the  $P_2O_5$  in ammoniated mixtures was determined by pot tests with two sets of samples. The values in Table 5 indicate that dolomite tends to reduce the availability of  $P_2O_5$  to a considerable extent in the mixtures stored at 60 and 90°C. and to a lesser extent in those stored at 20°C. The greater proportion of the results, however, showed the presence of dolomite to have no significant effect on the availability to plants of the  $P_2O_5$  in the samples stored at 20°C. Two series of tests, those with millet and sorghum, produced such drastic differences in results between the samples containing dolomite and those not contain-

TABLE 5.—*Effect of the presence of dolomite during ammoniation and storage on the availability to plants of the  $P_2O_5$  in ammoniated superphosphate mixtures.*  
Degree of ammoniation, 5 per cent; moisture 9 per cent

TEST MIXTURE	DOLOMITE	TEMPERATURE OF STORAGE	AVERAGE RELATIVE INCREASE IN YIELD <sup>1</sup>
No.		°C.	Per cent
17	Absent	20	75
5	Present	20	65
18	Absent	60	76
9	Present	60	55
19	Absent	90	72
13	Present	90	53

<sup>1</sup> On the basis of 100 as the average relative increase for the non-ammoniated mixture.

ing it, that the average of all results shows some reduction in availability of the samples containing dolomite when stored at 20°C. Table 5 also shows, that in mixtures without dolomite, the temperature of storage has little or no influence on the availability of  $P_2O_5$  to plants.

## EFFECT OF FLUORINE

The three superphosphates used in the preparation of the samples for this series of tests were prepared in the laboratory. One was a synthetic superphosphate consisting of a mixture containing one mol of C.P. mono-calcium phosphate to each 2 mols of calcium sulfate, with sufficient filler to reduce the  $P_2O_5$  content to 20 per cent. Three sets of 3 samples each were prepared from the synthetic superphosphate. One set contained no fluorine; another contained fluorine as calcium fluoride; and the third contained fluorine as sodium fluoride, the fluorine added in each case being equivalent to 10 per cent of the  $P_2O_5$  present. The second superphosphate was prepared from Tennessee phosphate rock containing 3.22 per cent fluorine, and the third from the same rock after it had been defluorinated.

One sample of each set (not ammoniated) was stored at 20°C.; a second



was ammoniated to 5 per cent and stored at 20°C.; and the third was ammoniated to the same degree and stored at 60°C.

The values shown in Table 6 indicate that the presence of added soluble fluorides has, on the average, little effect on the availability to plants of the  $P_2O_5$  in either the ammoniated or non-ammoniated mixtures containing synthetic superphosphate, but that some effect is apparent when the fluorine is present as superphosphate from Tennessee rock. Bartholomew (1) observed that soluble fluorine compounds added to the soil did not

TABLE 6.—*Effect of the presence of fluorine during ammoniation and storage on the availability to plants of the  $P_2O_5$  in ammoniated superphosphate mixtures. Dolomite absent; moisture 9 per cent*

TEST MIXTURE	FLUORINE	DEGREE OF AMMONIATION: NH <sub>3</sub> ADDED ON BASIS OF SUPERPHOSPHATE IN MIXTURE	TEMPERATURE OF STORAGE	AV. RELATIVE INCREASE IN YIELD <sup>1</sup>
No.		Per cent	°C.	Per cent
<i>Synthetic Superphosphate</i>				
26	Absent	0	20	100
27	Absent	5	20	75
28	Absent	5	60	80
29	Present as CaF <sub>2</sub>	0	20	100
30	Present as CaF <sub>2</sub>	5	20	94
31	Present as CaF <sub>2</sub>	5	60	71
32	Present as NaF	0	20	100
33	Present as NaF	5	20	89
34	Present as NaF	5	60	77
<i>Superphosphate from Tennessee Rock</i>				
35	Present	0	20	103
36	Present	5	20	63
37	Present	5	60	59
<i>Superphosphate from Defluorinated Tenn. Rock</i>				
38	Absent	0	20	100
39	Absent	5	20	83
40	Absent	5	60	82

<sup>1</sup> On the basis (test mixtures 26–34) of 100 as the average relative increase for the non-ammoniated synthetic superphosphate mixture and of 100 (test mixtures 35–40) as the average relative increase for the non-ammoniated superphosphate made from defluorinated Tennessee rock (No. 38).

affect the availability of phosphorus in the soil or that added as mono-calcium phosphate, but the presence of fluorine in naturally occurring phosphate rock greatly influenced the availability of their phosphorus to plants. MacIntire and coworkers (6) observed that no toxic effect on plant growth was caused "by the component fluorides of superphosphates used judiciously in relation to the need for liming."

#### SHORT-SEASON VERSUS LONG-SEASON CROPS

Long experience has shown that crops possess marked differences in their ability to acquire phosphorus from water-insoluble sources. This difference in the response that crops make to phosphates of relatively low

TABLE 7.—Percentage availability to short- and to long-season crops of the  $P_2O_5$  in ammoniated superphosphate mixtures. Dolomite present; moisture 9 per cent; temperature of storage 20°C.

TEST MIXTURE	DEGREE OF AMMONIATION: NH <sub>3</sub> ADDED ON BASIS OF SUPERPHOSPHATE IN MIXTURE	AVERAGE RELATIVE INCREASE IN YIELD	
		SHORT-SEASON CROPS <sup>1</sup>	LONG-SEASON CROPS <sup>2</sup>
No.	Per cent	Per cent	Per cent
1	0	100	100
2	2	98	106
3	3	84	101
4	4	76	106
5	5	55	98

<sup>1</sup> Millet, sorghum, and one cutting of Sudan grass.<sup>2</sup> Wheat and two cuttings of Sudan grass.

solubility varies with their growing season, their root development and other inherent genetic characteristics (3, 10).

In the present investigation the crops of millet, sorghum, and one cutting of Sudan grass were harvested on an average of about 55 days from the time of planting. The period between seeding time and harvest for wheat and two cuttings of Sudan grass amounted to about 125 days. Millet, sorghum, and one cutting of Sudan grass may thus be considered as short-season crops, while wheat and two cuttings of Sudan grass are relatively long-season crops. The relative increases in yield shown in Table 2 represent the average of the values found for all crops grown on the acid soils. In Table 7 the values found for these crops are broken down into the average relative increases in yield found for (a) the short-season crops: millet, sorghum, and one cutting of Sudan grass; and (b) the long-season crops: wheat and two cuttings of Sudan grass. The data in Table 7 are in agreement with the observation that, when other factors are the same, long-season crops are able to utilize water-insoluble phosphates to better advantage than those having a shorter growing season. The values in the table indicate that the ammoniation of a superphosphate, to 3 per cent or more, causes a significant decrease in the availability of its  $P_2O_5$  content to short-season crops. The results obtained with the long-season crops indicate, on the other hand, that there is no material difference between the efficiency of the non-ammoniated samples and any of the ammoniated samples that had been stored at 20°C. It is felt, however, that the data submitted are too limited to justify any definite conclusions on the relative response of long- and short-season crops to heavily ammoniated superphosphates.

#### CHEMICAL AVAILABILITY IN RELATION TO CROP RESPONSE ON ACID SOILS

In Table 8 the availability of the ammoniated superphosphate samples as determined chemically by the official method is compared with their

availability in promoting crop growth as indicated by pot tests on the acid soils. The results indicate that the official method can be used to evaluate accurately the availability to plants of the  $P_2O_5$  in non-ammoniated goods, and in ammoniated mixtures containing a maximum of about 2 per cent ammonia on the basis of the superphosphate present. The table shows, however, that in more heavily ammoniated mixtures the official method gives higher availability values than those indicated by the pot tests for all crops, and that the spread between the two methods increases with increase in the ammoniation of the mixture.

TABLE 8.—*Effect of various treatments on the availability of the  $P_2O_5$  in ammoniated superphosphate mixtures. Dolomite present; moisture 9 per cent*

TEST MIXTURE	DEGREE OF AMMONIATION	TEMPERATURE OF STORAGE	PERCENTAGE AVERAGE AVAILABILITY OF $P_2O_5$ IN SAMPLE		
			AS DETERMINED CHEMICALLY	AS DETERMINED BY POT TESTS ON ACID SOILS	
				ON BASIS OF 100 FOR NON-AMMONIATED SAMPLE	ON BASIS OF 95 FOR NON-AMMONIATED SAMPLE
No.	Per cent	°C.	ON BASIS OF A 1-GRAM SAMPLE <sup>1</sup>		
1	0	20	95	100	95
2	2	20	94	100	97
3	3	20	94	88	84
4	4	20	94	82	78
5	5	20	93	65	62
6	2	60	92	93	88
7	3	60	91	68	65
8	4	60	90	62	59
9	5	60	90	55	52
10	2	90	75	76	72
11	3	90	74	59	56
12	4	90	74	53	50
13	5	90	84	53	50

<sup>1</sup> Chemical availability determined on a 4-12-4 mixture after ammoniation and storage.

The principal phosphatic components of a superphosphate ammoniated to 2 per cent on the basis of 20%  $P_2O_5$  are monoammonium phosphate, dicalcium phosphate, and small amounts of monocalcium phosphate and undecomposed rock (2, 4). As the quantity of added ammonia is increased beyond 2.3 per cent, less soluble phosphate is formed at the expense of the more soluble phosphatic components of the mixture (4, 5). This less soluble phosphate may consist of one or more basic phosphates such as tricalcium phosphate, hydroxyl apatite, and more or less fluorinated apatites (6). In this report the term "reverted  $P_2O_5$ " will refer to the  $P_2O_5$  in these basic phosphates.

The increase in the spread of the results obtained by the two methods with increase in the rate of ammoniation is to be expected if it is assumed

that the percentage availability to plants of the reverted  $P_2O_5$  in heavily ammoniated superphosphates remains essentially the same irrespective of the degree of ammoniation. In the official chemical method for evaluating  $P_2O_5$  availability, the proportion of the reverted  $P_2O_5$  in heavily ammoniated superphosphates which dissolves in 100 ml of the neutral ammonium citrate solution does not remain the same, but varies with the proportion of the reverted  $P_2O_5$  in the sample taken for analysis.

TABLE 9.—*Per cent availability of the  $P_2O_5$  in ammoniated superphosphate mixtures. Dolomite present; moisture 9 per cent; temperature of storage 20°C.*

TEST MIXTURE	DEGREE OF AMMONIATION	AVAILABILITY FOUND BY CHEMICAL METHOD		AVAILABILITY BY POT TESTS; MEAN OF ALL CROPS	
		1-GRAM SAMPLE <sup>1</sup>	2-GRAM SAMPLE <sup>1</sup>	FOUND <sup>2</sup>	CALCULATED <sup>3</sup>
No.	Per cent	Per cent	Per cent	Per cent	Per cent
1	0	95	93	95	95
2	2	94	91	95	95
3	3	94	87	84	85
4	4	94	85	78	75
5	5	93	80	62	68

<sup>1</sup> As determined in a 4-12-4 mixture

<sup>2</sup> On basis of 95 as the availability for the non-ammoniated mixture.

<sup>3</sup> On assumption that the  $P_2O_5$  in the reverted phosphate is 50 per cent available to plants and in undecomposed rock, 15 per cent available.

If the proportion of reverted  $P_2O_5$  in a sample is small, the action of the citrate solution may be such as to dissolve completely the  $P_2O_5$  present, indicating complete availability. However, if the reverted  $P_2O_5$  in the sample is large, the citrate solution may then be capable of dissolving only a small proportion of the  $P_2O_5$  and a low availability will be indicated. This explains the action of the official method, already referred to, in giving a high availability rating for ammoniated mixtures of low  $P_2O_5$  content, and a low availability rating for mixtures of high  $P_2O_5$  content (2, 8).

According to Keenen (4) the reverted  $P_2O_5$  in an ammoniated superphosphate containing 20 per cent of total  $P_2O_5$  amounts to about 4.0 per cent of the sample at 3 per cent ammoniation, 8.2 per cent at 4 per cent ammoniation, and 12.0 per cent at 5 per cent ammoniation. The corresponding  $P_2O_5$  contents of ammoniated 4-12-4 mixtures prepared from the same superphosphate would be 2.4, 5.0, and 7.2 per cent, respectively.

If it be assumed that the  $P_2O_5$  in the reverted phosphate, and in the undecomposed rock, of ammoniated goods are respectively 50 and 15 per cent available to all crops on acid soils, then the availability to plants of the  $P_2O_5$  in the ammoniated samples may be calculated as shown by the data in Table 9. The table also shows the availability of the samples as found (1) by pot tests, and (2) by the official chemical method when (a) 1-gram, and (b) 2-gram samples are taken for analysis. The close agree-

ment between the calculated availability values and those found by the pot tests indicates that the percentage availability to plants of the reverted  $P_2O_5$  in heavily ammoniated superphosphate mixtures does remain essentially the same irrespective of the degree of ammoniation.

Table 9 further indicates that, as compared with pot tests on all crops with acid soils, the chemical method using a 2-gram sample for analysis may give slightly low results for non-ammoniated and 2 per cent ammoniated samples, and high results for those ammoniated in excess of 3 per cent. The official method in which a 1-gram sample is taken for analysis, also gives high results for heavily ammoniated mixtures but it can apparently be used to accurately evaluate non-ammoniated goods and ammoniated mixtures containing a maximum of 2 per cent of ammonia.

#### CROP RESPONSE ON ALKALINE SOIL

Two series of pot tests were conducted to determine the availability of  $P_2O_5$  in ammoniated superphosphate mixtures, to sorghum grown on an

TABLE 10.—*Effect of various treatments on the availability of the  $P_2O_5$  in ammoniated superphosphate mixtures to sorghum grown on alkaline soil.<sup>1</sup>*  
*Dolomite present; moisture 9 per cent*

TEST MIXTURE	DEGREE OF AMMONIATION	TEMPERATURE OF STORAGE	PERCENTAGE AVERAGE AVAILABILITY OF $P_2O_5$ IN SAMPLE	
			AS DETERMINED CHEMICALLY	AS DETERMINED BY POT TESTS
			ON A BASIS OF A 1-GRAM SAMPLE <sup>2</sup>	ON BASIS OF 100 FOR NON-AMMONIATED SAMPLE
No.	Per cent	°C.		
1	0	20	95	100
2	2	20	94	63
3	3	20	94	69
4	4	20	94	38
5	5	20	93	5
9	5	60	90	-13
13	5	90	84	+27

<sup>1</sup> Houston clay, pH 8.2.

<sup>2</sup> Chemical availability determined on a 4-12-4 mixture after ammoniation and storage.

alkaline soil. This soil was Houston clay with a pH of 8.2. There is evidence that heavily ammoniated superphosphates are less available to plants grown on alkaline soils than to those grown on acid soils (7, 9). It was considered desirable therefore not to include the data obtained on the Houston clay soil in the same tabulation with that reported for the acid soils.

Table 10 gives the results of various treatments on the availability of the  $P_2O_5$  in ammoniated superphosphate mixtures to sorghum grown on an alkaline soil. The data indicate that as little as 2 per cent ammoniation

of the superphosphate in the mixture decreases the availability of the  $P_2O_5$  to the crop grown on this soil. The data further indicate that the spread between the percentage average availability of the  $P_2O_5$  as determined chemically and by pot tests increases with increase in the degree of ammoniation, and with increase in temperature of storage after ammoniation. The results of these pot tests show that the  $P_2O_5$  in the ammoniated mixtures is considerably less available to plants grown on this alkaline soil than to those grown on acid soils.

#### SUMMARY

A collaborative study was made of the factors that affect the efficiency of ammoniated superphosphate mixtures in promoting crop growth in the greenhouse. The results of pot tests with 7 different acid soils in 5 separate locations indicate that:

- (a) Superphosphate mixtures that have been ammoniated to 2 per cent on the basis of the superphosphate in the mixture and stored at ordinary temperatures, with or without dolomite, are as available to plants on acid soils as the corresponding non-ammoniated mixtures.
- (b) The availability to plants of the  $P_2O_5$  in ammoniated mixtures containing dolomite tends to decrease as the rate of ammoniation is increased beyond 3 per cent, and as the temperature of storage is increased above normal. The presence of dolomite apparently causes a reduction in the availability to plants of the  $P_2O_5$  in ammoniated mixtures stored at elevated temperatures and, to a lesser extent, in such mixtures stored at ordinary temperatures.
- (c) The availability to plants of the  $P_2O_5$  in heavily ammoniated mixtures is less, for all three of the temperatures at which they were stored, when the moisture content of the mixture was 9 per cent than when it was 3 per cent.
- (d) The addition of fluorides to an ammoniated or a non-ammoniated mixture has little or no effect on the availability of its  $P_2O_5$  content to plants.
- (e) Long-season crops appear to be more responsive to water-insoluble phosphates than those having a shorter growing season.
- (f) The percentage availability to plants of the reverted  $P_2O_5$  in heavily ammoniated superphosphate mixtures remains essentially the same irrespective of the degree of ammoniation. As determined by the official chemical method, the percentage availability of the reverted  $P_2O_5$  in ammoniated mixtures does not remain the same, but varies with the proportion of the reverted  $P_2O_5$  in the sample taken for analysis. The spread between the percentage availability of the  $P_2O_5$  in ammoniated mixtures as determined chemically, and by pot tests, increases therefore as the degree of ammoniation is increased beyond 2 per cent.
- (g) Ammoniated superphosphates are less effective in promoting crop growth on alkaline than on acid soils.

## APPENDIX

SECTION 1.—*Effects of degree of ammoniation, temperature of storage, presence of dolomite, moisture and source of  $P_2O_5$  on response of millet, wheat, sudan grass, and sorghum to ammoniated superphosphates on various soils*

TEST MIX-TURE	SUPER-PHOS-PLATE	NH <sub>3</sub> ADDED	STOR-AGE TEMP.	H <sub>2</sub> O	DOLOMITE	YIELDS <sup>1</sup> OF VARIOUS CROPS ON INDICATED SOILS												AVERAGE RELATIVE INCREASES IN YIELD <sup>2</sup>
						MILLET <sup>3</sup>		WHEAT <sup>3</sup>		SUDAN GRASS <sup>4</sup>				SORGHUM				
						HART-BELLS	WOOSTER	WOOSTER	SASSA-PEAS	NEW-TONIA <sup>5</sup>	CECIL <sup>6</sup>		GRADY <sup>6</sup>	AT-WOOD <sup>4</sup>	AT-WOOD <sup>4</sup>	HOUS-TON <sup>4</sup>		
											A	B						
No.		per cent	°C.	per cent	lbs./ton	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	per cent	
Effect of Degree of Ammoniation																		
Check	None	—	—	—	—	0.7	14.4	9.3	0.7	3.9	0.9	0.0	10.8	22.1	15.8	15.7	—	
1	A	0	20	9	250	11.7	36.1	31.3	32.3	25.9	23.7	17.3	24.3	41.7	35.2	21.7	100	
2	A	2	20	9	250	11.0	39.3	34.2	33.2	26.2	24.9	17.2	24.5	39.3	32.1	19.3	100	
3	A	3	20	9	250	11.3	27.5	32.9	30.0	23.9	23.3	18.1	19.5	39.8	32.8	20.4	88	
4	A	4	20	9	250	11.2	25.1	33.5	31.9	20.2	23.1	21.0	21.6	37.0	26.6	17.5	82	
5	A	5	20	9	250	9.5	25.0	33.1	28.6	15.7	21.0	19.2	16.1	32.9	19.2	14.5	65	
Effect of Temperature of Storage (Tests 2-5 vs 6-9 and 10-13)																		
6	A	2	60	9	250	11.1	33.0	33.3	29.9	24.9	25.6	16.8	19.7	33.0			93	
7	A	3	60	9	250	8.6	27.1	31.4	29.9	16.2	21.0	20.3	16.5	22.1			68	
8	A	4	60	9	250	9.3	24.0	29.8	29.6	15.9	21.8	20.0	15.5	17.2			62	
9	A	5	60	9	250	7.0	20.6	30.4	30.1	12.9	18.2	16.5	15.2	18.1	14.2		55	
10	A	2	90	9	250	9.2	27.3	29.9	26.9	24.8	24.2	18.1	17.5	25.4			76	
11	A	3	90	9	250	7.6	25.0	30.0	26.1	14.4	18.9	17.3	14.5	21.3			59	
12	A	4	90	9	250	7.7	21.8	29.6	26.3	12.0	18.3	19.5	15.1	17.7			53	
13	A	5	90	9	250	8.2	20.8	30.5	28.0	9.0	18.1	19.7	15.3	32.4	19.0	13.3	53	

*Effect of Moisture (Tests 5, 9, and 13 vs 14, 15, and 16, respectively)*

14	A	5	20	3	250	10.0	26.9	32.8	32.9	19.2	23.1	18.7	19.2	37.3	24.2	14.0	78
15	A	5	60	3	250	8.7	28.8	31.7	30.4	14.3	22.1	18.7	15.8	27.6	18.8	14.3	66
16	A	5	90	3	250	8.9	26.0	30.3	27.0	19.6	20.7	16.5	21.5	35.8	26.7	18.0	75

*Effect of Dolomite (Tests 5, 9, and 13 vs 17, 18, and 19, respectively)*

17	A	5	20	9	none	9.8	27.6	33.5	28.3	15.3	20.2	19.9	17.1	27.4			75
18	A	5	60	9	none	8.6	22.6	30.5	30.1	15.7	22.0	17.7	22.1	24.1			76
19	A	5	90	9	none	8.0	26.2	30.8	30.8	18.1	22.0	17.1	15.5	25.3			72

*Effect of Source of P<sub>2</sub>O<sub>5</sub> (Test 1 vs 20, 22, or 24 and test 8 vs 21, 23, or 25)*

20	B	0	20	9	250	11.7		32.6	34.3	27.5	40.5 <sup>a</sup>	17.0	28.5	38.0			110
21	B	4	60	9	250	8.0		31.5	28.9	9.3	26.4	17.3	18.2	16.2			70
22	C	0	20	9	250	11.8		33.2	35.6	27.2	37.2	15.1	27.1	36.5			109
23	C	4	60	9	250	8.2		30.4	31.5	9.7	25.4	17.1	17.4	21.2			67
24	D	0	20	9	250	12.3		32.0	35.2	28.4	38.5	15.9	24.5	40.9			106
25	D	4	60	9	250	9.2		31.7	33.2	17.1	30.4 <sup>a</sup>	16.6	17.3	21.7			78

Difference required for significance:<sup>a</sup>

5% level	3.3	5.4	2.5	4.7	6.2	10	4.1	6.1	7.6	3.8
1% level	4.4	7.2	3.4	6.2	8.2		5.5	8.2	10.0	5.0

<sup>1</sup> Average weight per pot of oven-dried aerial portions of crop harvested a little before maturity.<sup>2</sup> Moisture content during ammoniation and storage.<sup>3</sup> The fertilizer was mixed with 5 per cent of the soil and placed in a horizontal layer half way between the bottom and top of the pot.<sup>4</sup> The fertilizer was placed in a circular area 3 inches in diameter and 1.5 inches from the top of the soil. The seed were planted in a 5-inch ring concentric with this smaller area.<sup>5</sup> First cutting.<sup>6</sup> First cutting (A). Second cutting (B).<sup>7</sup> On the basis of 100 as the average increase for the non-ammoniated superphosphate; results on alkaline soil not included.<sup>8</sup> Planted a month later than other members of the series.<sup>9</sup> Between treatments but not between treatments and checks.<sup>10</sup> Individual pot yields not reported.



## APPENDIX

SECTION 2.—Effect of presence of fluorine during ammoniation and storage on response of crops to ammoniated superphosphates on various soils<sup>1</sup>

TEST MIXTURE	NH <sub>3</sub> ADDED	STORAGE TEMP.	YIELDS <sup>2</sup> OF VARIOUS CROPS ON INDICATED SOILS									AVERAGE RELATIVE INCREASE <sup>3</sup>
			MULLY <sup>4</sup>	WHEAT <sup>5</sup>		SUDAN GRASS <sup>4</sup>			SORGHUM <sup>5</sup>			
				HARTBELLS	WOOSTER	SASSAFRAS	NEWTONIA	CECIL	GRADY	ATWOOD		
No. Check	per cent —	°C. —	gms. 4.5	gms. 9.3	gms. 0.7	gms. 3.9	gms. 0.9	gms. 10.8	gms. 15.8	per cent —		
<i>Synthetic Superphosphate, Fluorine free</i>												
26	0	20	27.3	35.8	33.9	28.5	26.3	23.2	35.4	100		
27	5	20	21.2	34.4	33.6	15.3	22.3	17.1	31.5	75		
28	5	60	26.2	35.0	35.8	16.8	28.7	16.5	27.1	80		
<i>Synthetic Superphosphate, Fluorine added as CaF<sub>2</sub></i>												
29	0	20	27.4	36.6	38.0	26.3	25.2	23.7	33.9	100		
30	5	20	26.8	33.9	37.6	22.2	25.9	23.1	30.4	94		
31	5	60	20.1	29.8	28.5	18.5	25.5	18.9	25.4	71		
<i>Synthetic Superphosphate, Fluorine added as NaF</i>												
32	0	20	26.6	32.9	35.7	27.0	29.9	21.8	38.0	100		
33	5	20	25.6	33.8	36.4	26.3	25.5	18.5	32.5	89		
34	5	60	20.5	33.8	32.3	16.3	22.4	22.0	27.2	77		

*Superphosphate made from Tennessee Rock Phosphate*

	35	0	20		29.6	30.2	28.7	37.6 <sup>a</sup>	22.3	37.6	103
36	5	20			29.7	29.3	14.2	27.5	17.8	19.4	63
37	5	60			28.6	29.1	15.8	28.9	13.9	20.6	89
<i>Superphosphate made from Defluorinated Tennessee Rock Phosphate</i>											
38	0	20			33.9	35.0	24.8	38.4	20.5	34.3	100
39	5	20			33.0	31.7	19.6	33.2	18.9	29.0	83
40	5	60			31.5	32.9	14.4	36.8 <sup>a</sup>	21.6	25.5	82
<i>Difference Required<sup>7</sup></i>											
	5% level				1.7	4.7	6.2	4.1	7.6		
	1% level				2.3	6.2	8.2	5.5	10.0		

<sup>1</sup> All mixtures contained 9 per cent of water and dolomite was absent.<sup>2</sup> Average weight per pot of oven-dried aerial portions of crop harvested near maturity.<sup>3</sup> The fertilizer was mixed with 5 per cent of the soil and placed in a horizontal layer half way between the bottom and the top of the pot.<sup>4</sup> The fertilizer was placed in a circular area 3 inches in diameter and 1.5 inches from the top of the soil. The seed were planted in a 5-inch ring concentric with this fertilized area.<sup>5</sup> On the basis (test mixtures 26-34) of 100 for the non-ammoniated fluorine-free synthetic superphosphate (No. 26) and of 100 (test mixtures 35-40) for the non-ammoniated superphosphate made from defluorinated rock (No. 38).<sup>6</sup> Planted a month later than other members of the series.<sup>7</sup> Between treatments but not between checks and treatments.

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THE AIR-FLOW METHOD FOR THE DETERMINATION  
OF MOISTURE IN FERTILIZERS\*

By JOHN O. HARDESTY, COLIN W. WHITTAKER,  
and WILLIAM H. ROSS<sup>1</sup>

(Division of Fertilizer and Agricultural Lime, Bureau of Plant Industry,  
Soils, and Agricultural Engineering, Agricultural Research  
Administration, U. S. Department of Agriculture,  
Beltsville, Maryland)

Water in fertilizer materials and mixtures may exist as free moisture; water of crystallization, such as that occurring in gypsum,  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ; or water of constitution as in phosphoric acid or sundry organic materials that are often components of mixed fertilizers. The free moisture and its burden of dissolved salts forms a liquid phase on the surface of the solid particles and determines, to a large extent, the physical condition of the fertilizer. An accurate knowledge of the free moisture content is thus of prime importance to the fertilizer manufacturer and chemist. Unfortunately, such knowledge is not always afforded by methods commonly used.

In a review of the problem of determining moisture in fertilizers, Whittaker and Ross (4) pointed out that the decomposition of organic materials and of crystalline hydrates and the liberation of water of constitution, that may occur when samples are dried at  $100^\circ\text{C}$ ., could be largely avoided if the moisture were removed rapidly at a low temperature as by passing a current of air, heated to about  $60^\circ\text{C}$ ., directly through the sample. Such a technique would take advantage of the slower rate of liberation of water of crystallization and of constitution, than of free moisture, and of the greater stability of crystal hydrates and

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<sup>1</sup> Deceased.

organic materials at the lower temperature. These workers also indicated that drying over a suitable desiccant for at least two hours at room temperature in a well evacuated desiccator approached the ideal procedure more closely than most of the other methods studied. However, some of the commonly used powerful drying agents like anhydrous magnesium perchlorate are capable of reducing the partial vapor pressure of moisture surrounding the sample to such an extent that some of the crystalline hydrates like magnesium ammonium phosphate hexahydrate, that may be present in mixed fertilizers, may lose water of crystallization. Three essentials of a method for free moisture would thus appear to be (a) rapid removal of the moisture, (b) low temperature, and (c) a not too low partial vapor pressure of water vapor in the atmosphere surrounding the sample. The present official method (2) for determining moisture by oven drying for 5 hours at 100°C. does not meet any of the foregoing conditions. The present paper describes a method, designated as the air-flow method, in which the necessary conditions are met more adequately.

Elsewhere in this issue (3) Ross and Love have compared the air-flow method with the official method and the vacuum-drying procedure for the determination of moisture in various types of fertilizer materials. They found that the principal salt hydrates that are likely to occur in mixed fertilizers can be dried by the air-flow method without the loss of water of crystallization that occurs when the same materials are dried by the official method. The present paper extends these results to mixed fertilizers with special attention to those that may lose water of constitution at 100°C. or in which thermal reactions generating volatile reaction products may occur.

#### AIR-FLOW METHOD

##### *Apparatus*

The equipment designed for the rapid removal of moisture at a relatively low temperature is shown in Figures 1 and 2. It consists of a metal manifold (Figure 1)  $10\frac{1}{2} \times 2\frac{1}{2} \times 1\frac{1}{4}$  inches in size with a  $\frac{1}{4}$ -inch nipple centrally located on one side for attachment to the vacuum line, and six  $1\frac{1}{4}$ -inch tapered stopper seats evenly spaced along the top to accommodate a No.-6, one-hole, rubber stopper. A  $1\frac{1}{4}$ -inch length of light metal tubing,  $\frac{1}{2}$ -inch in diameter, extends through the rubber stopper to a height of approximately  $\frac{1}{4}$ -inch above the surface. This extension of the metal tubing above the surface of the stopper is for the purpose of centering a fritted glass crucible over the hole in the stopper. Since the crucible is held in place by suction, it is necessary to grind a smooth surface on the lower edge of each fritted glass crucible and the surface of the stopper in order to insure an air-tight connection between the edge of the crucible and the stopper when air is being drawn through the sample in the crucible. Figure 2 shows the manifold with crucibles in position in a constant temperature oven and connected to a gage in the vacuum line. The most suit-

able type of drying oven for the determination is one in which the incoming air is drawn over the heating coils before it contacts the samples. With this equipment the amount of air passing through the sample and the temperature of the sample can be maintained constant. A manifold to accommodate 12 or more crucibles may be used providing sufficient vacuum is maintained to keep approximately the same quantity of air passing through each sample.

The fritted glass crucible is the type ordinarily used for filtration. Those

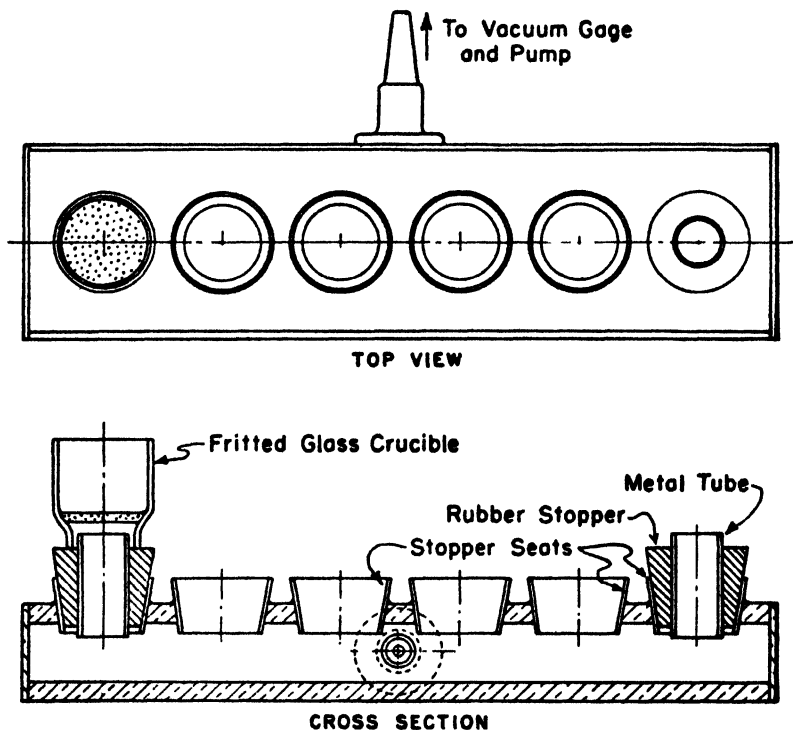


FIG. 1.—Manifold for use in determining moisture by the air-flow method.

used in this work were of Pyrex glass,  $1\frac{1}{2}$  inches tall,  $1\frac{3}{8}$  inches in diameter at the top and fitted with a  $\frac{1}{8}$ -inch fine-porosity fritted glass plate. For accurate work a set of crucibles of matched porosity should be used. Such a set may be obtained by selecting several that pass a given quantity of air at a constant pressure in about the same length of time. For this purpose the rate of air flow may be determined with a gas meter or by fitting the crucible to the tight-fitting rubber stopper of a large separatory funnel filled with water and selecting crucibles which give approximately the same length of time for the funnel to empty.

Tests have shown that matched fine-porosity crucibles under constant

vacuum allow the passage of air through each crucible on the manifold at approximately the same rate (0.1 cu. ft. in 60–108 seconds at 60°C. and 63 cm. vacuum), and, since the resistance to air flow afforded by the fritted disk is large relative to that afforded by the sample, variations in

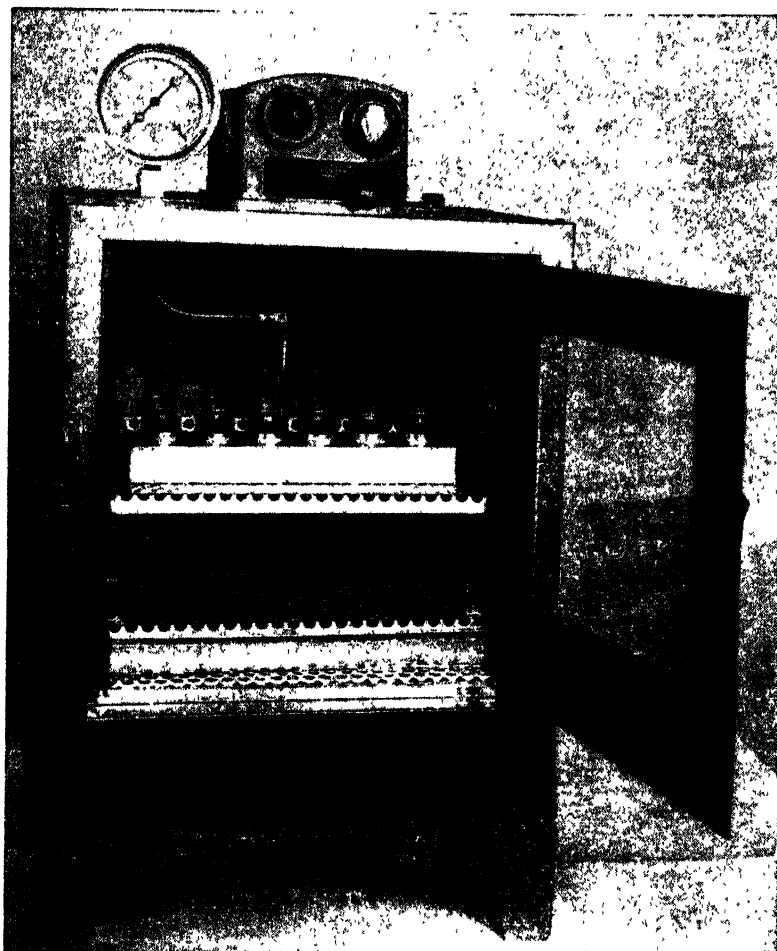


FIG. 2.—The air-flow apparatus in place in a drying oven.

the amount and particle size of the sample have no appreciable effect on the amount of air passed through the sample in a given length of time. A vacuum of about  $\frac{1}{2}$  atmosphere has been found suitable for most moisture determinations. This facilitates routine procedure in that all samples may be removed at the same time with the reasonable assurance that they have been subjected to the same drying conditions.

### *Procedure*

A two-gram sample of the fertilizer or fertilizer material is weighed into tared crucible and spread evenly over the fritted glass disk. When six crucibles (or the manifold capacity) have been prepared and the oven temperature adjusted to 60°C., the vacuum is turned on the manifold and the crucibles are put in place on the rubber stoppers, making certain that each is tightly seated as indicated by its resistance to removal. If less than six crucibles are to be used the other openings in the manifold must be tightly stoppered. The vacuum is adjusted to about  $\frac{1}{2}$  atmosphere and the air flow continued for two hours, or longer if necessary. The oven thermometer should be located with its bulb near the top of the manifold to insure that the temperature of the air entering the crucibles is being measured. The oven temperature should be checked a few minutes after the vacuum is turned on and again later. Some ovens do not readily take up the additional load because of the cool air being drawn in, so that adjustment is sometimes necessary. The determinations usually proceed smoothly and few precautions are necessary. The fact that the air passes downward through the sample prevents any loss of fine particles through dusting. Loss in weight is computed after cooling the crucibles in a desiccator and weighing.

### APPLICATION TO MIXED FERTILIZERS

#### *Loss of Volatile Matter Other Than Free Moisture*

In a previous paper (1) it was shown that fertilizer mixtures containing superphosphate, inorganic nitrates, and organic matter may undergo decomposition at 100°C. This is the result of the oxidation of organic matter by nitric acid, which is formed in the reaction between nitrate and monocalcium phosphate, or free phosphoric acid, in the presence of moisture. Such mixtures heated at temperatures of 85 to 100°C. for 2 hours were found to lose between 6 and 7 per cent of their weight in the form of volatile matter, including oxides of nitrogen, carbon dioxide, and water of constitution. While these reactions may occur slowly at much lower temperatures, they are greatly accelerated at temperatures in the neighborhood of 100°C. No appreciable loss of volatile matter other than free moisture was observed to occur below 85°C. Therefore it would be expected that moisture determinations on such mixtures would give widely variant results depending on whether the determinations were made above or below 85°C. Mixtures were prepared and analyzed for moisture by 3 different methods according to the formulation and analytical data presented in Table 1. The mixtures were formulated to contain approximately 7 per cent free moisture according to analysis of the materials by vacuum desiccation; free moisture was added when necessary. At this moisture content the mixtures containing large amounts of am-

monium nitrate were slightly clammy after 30 days of storage in moisture-proof containers, while the other mixtures were dry and friable. Gross weight of the samples remained constant during storage, showing that no loss by evaporation of moisture occurred. During the moisture determination by the official method at 100°C., mixtures containing large amounts of ammonium nitrate in conjunction with unammoniated superphosphate

TABLE 1.—*Effect of fertilizer formulation on moisture values by different methods*

MATERIAL	FORMULAS OF FERTILIZER MIXTURES (POUNDS PER TON)						
	8-24-0	8-24-8			4-24-8	3-12-6	8-16-16
	No. 1	No. 5	No. 7	No. 9	No. 13	No. 47004	No. 957
Ammonium Sulfate	385	385	—	385	385	—	310
Ammonium Nitrate	240	240	480	240	—	—	67
Nitrogen Solution 2A	—	—	—	—	—	171	190
Triple Superphosphate	1000	1000	1000	1000	1000	—	690
Normal Superphosphate	—	—	—	—	—	1200	—
Potassium Chloride	—	270	270	—	270	200	530
Potassium Sulfate	—	—	—	310	—	—	—
Hydrated Lime	25	25	25	25	25	—	—
Magnesium Limestone	—	—	—	—	—	116	113
Cocoa Shell Meal	100	80	100	40	100	—	100
Sand Filler	250	—	125	—	220	313	—
METHOD	MOISTURE VALUES AFTER CURING (PER CENT)						
	SAMPLE NUMBERS						
	1	5	7	9	13	47004	957
A.O.A.C. (5 hrs., 100°C.)	10.81	9.93	14.83	8.79	7.19	6.86	7.30
Vacuum Dessication <sup>1</sup>	4.48	4.73	7.18	4.48	2.23	6.04	5.92
Air-Fow (2 hrs., 60°C.) <sup>2</sup>	4.26	4.36	7.07	4.24	2.07	5.89	5.83

<sup>1</sup> With 27 inches vacuum, over anhydrous magnesium perchlorate for 48 hours at 25°C.

<sup>2</sup> Aspiration with 15 inches vacuum on manifold containing 6 samples in matched fine-porosity crucibles.

and organic matter (Nos. 1, 5, 7, and 9) had a marked tendency to liberate more volatile matter than that represented by the free moisture content (7 per cent) of the original mixture. They had an appreciable odor of nitric acid, and mixture No. 7 liberated noticeable brown fumes during the early stage of heating. This loss of volatile matter other than free moisture is reflected in the values obtained by the official method on mixtures 1, 5, 7, and 9. As compared with No. 7 containing no sulfate, mixtures 1, 5, 9, 13, and 957, containing large amounts of ammonium sulfate, gave evidence of hydrate formation as indicated by their low free moisture content according to the vacuum-drying and the air-flow methods of analysis. The results obtained by these two methods on mixture No. 7 show that little or no hydration occurred since the amount of free mois-



ture recovered after curing was essentially the same as the original free moisture content (7 per cent) of the sample.

The vacuum procedure gave results on all mixtures that were consistently higher, by small amounts, than those obtained by the air-flow method. This may be attributed to a slight, partial loss of water of crystallization when using the vacuum method, and suggests that the air-flow method gives a slightly better estimate of free moisture when crystal hydrates are present.

The results given in Table 1 demonstrate the applicability of the air-flow and vacuum-drying methods to the problem of following the course

TABLE 2.—*Loss in weight of cocoa shell meal during drying by different methods*

METHOD OF DRYING	DURATION OF DRYING	LOSS IN WEIGHT
	<i>Hours</i>	<i>Per cent</i>
Oven at 100°C.	3	6.29
" " "	5	6.49
" " "	7	6.60
Vacuum Drying <sup>1</sup>	16	3.28
" "	21	4.05
" "	26	4.22
Air-Flow <sup>2</sup>	1	3.57
" "	2	3.59
" "	3	3.59

<sup>1</sup> Over anhydrous magnesium perchlorate at 30°C.

<sup>2</sup> At 60°C.

of hydration reactions in fertilizers during the curing stage. They also indicate that the present official method of drying for 5 hours at 100°C. is not applicable to mixtures containing large amounts of ammonium nitrate in conjunction with organic matter and superphosphate.

### *Effect of Atmospheric Humidity*

The tenacity with which water is held by some organic fertilizer conditioners, *e.g.*, cocoa shell meal, is well known. With materials of this kind it would be expected that the partial pressure of moisture in the air passing through the sample would influence results by the air-flow method. A comparison of the results obtained by different methods of drying cocoa shell meal (Table 2) shows that oven drying at 100°C. gives considerably higher values than either the vacuum-drying or the air-flow method. This is attributed to the slow decomposition of the organic matter at this temperature. The data also show that drying in vacuum over anhydrous magnesium perchlorate for 21 to 26 hours gives somewhat higher values for moisture in this material than were obtained by the air-flow procedure.

When the material dried over the desiccant was further treated by the air-flow method, it regained an amount of moisture equivalent to the difference between the original results obtained by these two methods. This indicates that the moisture vapor in the air drawn through this highly adsorptive material prevented as complete drying as was obtained by the vacuum-drying procedure.

The relative humidity of the air in the laboratory during the course of experiments described in this paper varied between 23 and 70 per cent. However, with the exception of the cocoa shell meal and the phosphatic materials containing free phosphoric acid as described in the accompanying paper by Ross and Love (3), it will be observed (Table 1) that the results obtained by the air-flow method are in fair agreement with those obtained by the vacuum-drying method in which the humidity of the air in the laboratory is not a factor.

Known methods for determining moisture in fertilizer materials and mixtures are necessarily empirical when considered from the standpoint of their application to many types of samples. When extremely small changes in moisture content are of interest, as in the control analysis of ammonium nitrate, it may be expedient to either dry the air which passes through the sample during the determination of moisture by the air-flow method, or to maintain the air at a selected constant relative humidity. This would also offer the possibility of shortening the time required for the determination. However, the feasibility of modifying the method, to include such practice for routine analytical work on fertilizers, depends upon whether or not extreme accuracy is desired. The fertilizer chemist is interested in obtaining moisture results that reveal the causes for variation in either the physical condition of the fertilizer or in the results of analyses for other constituents. Possibly no single method is sufficient for all materials and mixtures. The present official methods are not applicable to many of our present day mixtures and it would seem necessary to replace or supplement them with more reliable methods. The air-flow and vacuum-drying methods show considerable promise in this respect since neither of them causes any appreciable decomposition of the organic matter or hydrates commonly occurring in fertilizers.

#### SUMMARY AND CONCLUSIONS

A procedure designated as the air-flow method of determining free moisture in fertilizer materials and mixtures is described. It consists briefly in drawing air heated to 60°C. through the sample for 2 hours.

Comparative results were obtained on fertilizer samples containing easily oxidizable components using three different methods of determining moisture, namely: the present official method in which the sample is oven dried for 5 hours at 100°C., the method of drying in vacuum over anhydrous magnesium perchlorate for varying periods of time at room

temperature, and the air-flow method. The results show that the present official method is not adapted to the determination of free moisture in such samples, since a temperature of 100°C. may cause decomposition and consequent loss in weight other than that representing the true free moisture content. These decomposition reactions do not occur during the determination by either the vacuum-drying or air-flow methods, which offer considerable promise for routine evaluation of free moisture in fertilizers.

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### A COMPARISON OF COPPER SULFATE AND MERCURIC OXIDE AS CATALYST IN THE DETERMINATION OF PROTEIN IN FISH MEAL

By THOMAS J. POTTS, MARY A. PARHAM, and IRMA M. SCHAFER  
(Ralston Purina Company, St. Louis, Mo.)

Several different catalysts have been used for the measurement of protein in feeding stuffs by the Kjeldahl procedure. Among those used are copper, mercury, and selenium. It has been observed by several laboratories that the use of copper as a catalyst resulted in lower protein content in vegetable oil meals than when mercury was used as the catalyst (1) (2).

In our laboratory we have used copper sulfate as a catalyst in nitrogen digestions for protein determinations for more than thirty years. In this report we are presenting data in which we compared the use of mercuric oxide with copper sulfate as a catalyst in the determination of protein in fish meal.

First, we investigated the effect of the kind of catalyst used on the minimum time required for complete digestion. Twenty-eight 1-gram portions of a fish meal sample were weighed into digestion flasks. To fourteen of them, 11 grams of anhydrous sodium sulfate, 0.2 gram anhydrous copper sulfate, and 25 ml of sulfuric acid were added. To the remainder, 11 grams of anhydrous sodium sulfate, 0.7 gram red mercuric oxide, and 25 ml of sulfuric acid were added.

Two portions were digested for each catalyst, and for each of the periods of time shown in Table 1.

The balance of the official procedure was then followed (3) (4). In the case of copper sulfate the solution was made alkaline with sodium hydroxide and distilled into N/5 sulfuric acid. In the case of mercuric oxide,

sodium thiosulfate, and sodium hydroxide were added before distillation according to the Kjeldahl-Gunning-Arnold procedure. The resulting ammonia was in every case distilled into the same quantity of N/5 sulfuric acid.

The work was repeated with five additional samples of fish meal.

TABLE 1.—*Comparison of digestion time for copper and mercury catalysts*

SAMPLE	TIME IN MINUTES AFTER CLEARING						
	20	40	60	80	100	120	140
	<i>Per cent</i> †	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
	(a) <i>Using Copper Sulfate</i> *						
B5610	65.00	65.72	65.77	65.85	65.80	65.75	66.03
B5634	57.96	59.50	59.62	59.59	59.59	59.66	59.59
B5638	63.42	64.60	64.59	64.63	64.65	64.66	64.66
B5646	67.17	68.15	68.04	68.22	68.41	68.24	68.34
B5670	62.51	63.42	63.77	63.86	63.82	63.79	63.70
B5680	64.44	65.68	65.54	65.63	65.52	65.70	65.52
Average	63.41	64.51	64.56	64.63	64.63	64.63	64.64
	(b) <i>Using Mercuric Oxide</i> *						
B5610	65.12	65.77	65.72	65.75	65.77	65.80	65.86
B5634	58.24	59.74	59.71	59.76	59.62	59.92	59.74
B5638	63.53	64.59	64.93	64.65	64.96	64.73	64.59
B5646	67.06	68.18	68.36	68.17	68.31	68.24	68.39
B5670	62.79	63.88	63.81	63.96	63.84	64.03	64.07
B5680	64.70	65.72	65.64	65.57	65.64	65.52	65.77
Average	63.57	64.65	64.70	64.64	64.69	64.71	64.74

\* Results are average of two determinations.

† Protein protein ( $N \times 6.25$ )

Total digestion time for:

CuSO<sub>4</sub> = 35 minutes + digestion time in table

HgO = 20 minutes + digestion time in table.

After determining the minimum digestion time, the information was used in determining the protein in forty samples of fish meal.

Four 1-gram portions of each sample were weighed out at the same time to make certain that comparisons were at the same moisture basis. Duplicate determinations were made using copper sulfate for a catalyst, and also duplicates using mercuric oxide. Results showing the average of these duplicate determinations are given in Table 2.

#### OBSERVATIONS

The results recorded in Table 1 show that the minimum time required for complete digestion is 40 minutes after the solution becomes clear. This time was used for the samples analyzed in Table 2. The data show that continued digestion up to 140 minutes does not cause any appreciable change.

TABLE 2.—*Per cent protein in fish meal*

SAMPLE NUMBER	COPPER SULFATE	MERCURIC OXIDE	COMPARISON OF Hg WITH Cu PER CENT PROTEIN
B5568	64.27	65.18	+0.91
B5569	63.56	64.34	+0.78
B5570	63.66	64.65	+0.99
B5581	66.35	66.37	+0.02
B5596	63.62	65.93	+2.31
B5597	64.80	64.89	+0.09
B5707	66.18	66.03	-0.15
B5708	60.63	61.29	+0.66
B5781	63.27	64.00	+0.73
B0014	70.31	70.81	+0.50
B0015	53.79	54.08	+0.29
B0116	65.10	66.08	+0.98
B0154	61.46	61.83	+0.37
B0132	59.25	59.62	+0.37
B0133	62.40	62.61	+0.21
B0188	69.17	69.03	-0.14
B0270	68.48	68.38	-0.10
B0328	68.74	68.73	-0.01
B0330	65.73	65.84	+0.11
B0331	69.87	71.62	+1.75
B0350	63.06	63.39	+0.33
B0383	63.29	63.61	+0.32
B0362	61.44	62.66	+1.22
B0464	64.05	65.07	+1.02
B0465	62.81	63.13	+0.32
B0475	59.39	59.45	+0.06
B0493	65.18	65.23	+0.05
B0547	63.72	64.34	+0.62
B0563	63.41	63.84	+0.43
B0729	61.56	61.76	+0.20
B0658	63.13	63.78	+0.65
B0733	59.85	59.78	-0.07
B0728	56.20	56.98	+0.78
B0848	67.61	68.21	+0.60
B0849	65.62	65.62	0.00
B0850	65.07	66.28	+1.21
B0865	68.74	69.07	+0.33
B0866	65.21	65.37	+0.16
B0953	60.95	64.74	+3.79*
B1044	67.09	67.09	0.00
Average	63.95	64.52	+0.57

\* This sample was normal in physical appearance.

It was noted in all these determinations that the copper digestion cleared after approximately 35 minutes and the mercury after 20 minutes.

The digestions were heated with open 500-watt electric heating elements.

In reviewing the results of Table 2 it will be noted that in the 40 samples analyzed all gave higher results with mercury except five. These samples show results from 0.01–0.15 per cent lower. The difference is so small that it is well within the experimental error.

Of the remaining 35 samples, 19 are from 0–.50 per cent higher for mercury, 10 are .50–1.00 per cent higher, 4 from 1.00–2.00 per cent higher, 1 is 2.31 per cent higher, and 1 is 3.79 per cent higher.

The average protein of these 40 samples is 0.57 per cent higher with mercuric oxide than with copper sulfate.

### DISCUSSION

The results secured with fish meal are in agreement with those of other protein concentrates investigated in this laboratory. Following the same chemical procedure the average difference obtained for 40 samples of soybeans and soybean meal was +0.25 per cent in favor of mercury. In 40 samples of cottonseed meal it was +0.23 per cent. In 20 samples of tankage it was +0.28 per cent, and in 40 samples of meat scrap +0.36 per cent. Thus, of the 719 determinations made in comparing these catalysts, the results secured with fish meal are the most impressive.

### CONCLUSIONS

Our observations indicate that when mercuric oxide is used as a catalyst results tend to be higher than when 0.2 gram anhydrous copper sulfate and 11 grams of sodium sulfate are used to determine protein in fish meal.

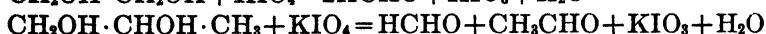
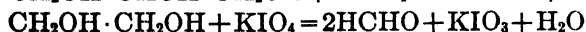
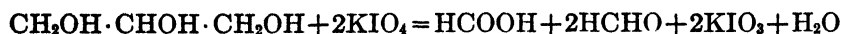
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## THE ESTIMATION OF GLYCEROL IN THE PRESENCE OF PROPYLENE AND ETHYLENE GLYCOLS

By S. H. NEWBURGER and C. F. BRUENING (Food and Drug Administration, Federal Security Agency, Baltimore, Maryland)

Shupe<sup>1</sup> utilizes the Malaprade periodate oxidation to determine glycerol in the presence of propylene and ethylene glycols.



<sup>1</sup> Shupe, I. S. *This Journal*, **26**, 249 (1943).

Since formic acid from glycerol is the only acid oxidation product yielded by any of these substances, a titration of the acid furnishes a method of determining glycerol. Each mol of glycerol provides one mol of formic acid.

Shupe makes the following recommendations:

(a) That appropriate correction for any acidity in the oxidizing 0.02 *M* potassium periodate solution be made.

(b) That the oxidized solution be titrated to a clear yellow end point with 0.02 *N* sodium hydroxide and methyl red indicator.

We found these recommendations difficult to follow. Potassium periodate solutions when titrated with 0.02 *N* sodium hydroxide and methyl red indicator do not show a sharp end point, but change slowly from a red to a salmon yellow color. On further addition of alkali, it is not possible to obtain the yellow color which is observed in the titration of the oxidized glycerol solutions. Nine collaborators<sup>2</sup> who determined glycerol in a vanishing cream reported blanks from 0.05 to 0.3 ml of 0.02 *N* sodium hydroxide for equal volumes of the same potassium periodate solution.

When oxidized glycerol solutions are titrated, the color changes from red, through a salmon yellow, to a yellow. However, the change from salmon yellow to clear yellow is so gradual that it is extremely difficult to ascertain when the end point has been reached. Experiments on solutions of known glycerol content have convinced us that the correction for the acidity of the potassium periodate must be omitted, and the titration continued until no perceptible color change is caused by the addition of one drop of 0.02 *N* sodium hydroxide. Otherwise, the results will be low.

The aforementioned collaborators<sup>2</sup> obtained recoveries averaging 100 per cent of the glycerol present in the vanishing cream when calculated from the periodate consumed, but their recoveries averaged only 96.4 per cent calculated from alkali titration of the same solutions. The individual results varied from 93.1 to 99.8 per cent.

The following observations provided the experimental basis for a modified method:

Potassium periodate solution is acid to methyl red, and the addition of 0.02 *N* sodium hydroxide produces a gradual color change. The solution never becomes a clear yellow color. Likewise, potassium iodate solution is acid to methyl red but changes sharply to a clear yellow on the addition of 0.05 ml of 0.02 *N* sodium hydroxide. That the poor end point with methyl red is probably due to the acidic properties of the potassium periodate itself was demonstrated in the following manner:

To a large excess of propylene glycol in aqueous solution were added a few drops of methyl red indicator and enough 0.02 *N* sodium hydroxide to make the solution a clear yellow. When potassium periodate was added

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<sup>2</sup> Bruening, C. F., *This Journal*, 30, 507 (1947).

the solution turned red. However, as the periodate was reduced to iodate by the propylene glycol, the solution reverted to a yellow color.

The above experiments indicated that if excess potassium periodate were destroyed by propylene glycol, before attempting the titration of formic acid, better results would be obtained in the alkalimetric determination of glycerol.

An effort was also made to substitute for methyl red an indicator showing a readily detected end point. Both bromcresol purple ( $pH$  5.2–6.8) and chlorphenol red ( $pH$  4.8–6.4) seemed to be more satisfactory than methyl red. Bromcresol purple was chosen since its color change is more easily seen.

The following modified method was developed:

#### REAGENTS

*Bromcresol purple indicator soln.*—Dissolve 0.1 gram in 100 ml of alcohol.

*Potassium periodate.*—0.02  $M$ . Dissolve 4.6 grams  $KIO_4$  in about 500 ml hot water. Dilute to about 900 ml with water, cool to room temperature, and make to 1000 ml.

*Sodium hydroxide.*—0.02  $N$ . Dilute 1 volume of 0.1  $N$   $NaOH$  to 5 volumes with water.

*Propylene glycol.*—Dilute 0.5 ml of commercial product to 25 ml with  $H_2O$ . Add 25 ml of 0.02  $M$   $KIO_4$  and allow to stand for 10 minutes. Titrate with 0.02  $N$   $NaOH$  using 3 drops of the indicator soln. If not more than 0.05 ml of the base is consumed, the product is suitable for use.

#### PROCEDURE

Transfer a sample containing not more than 45 mg of glycerol, or its equivalent, to a 110 ml volumetric flask. Add 1 drop of bromcresol purple indicator solution and neutralize. Make the final adjustment to a light purple color with 0.02  $N$   $NaOH$ . Add 50 ml of 0.02  $M$   $KIO_4$ , dilute to volume with water, mix and allow to stand for one hour.\* Pipet a 50 ml aliquot into a flask, add 10 drops of propylene glycol (ca 0.5 ml), mix well, wash down sides of flask with water, and allow to stand for 10 minutes. Add 3 drops of indicator and titrate with 0.02  $N$   $NaOH$  to a light purple end point. The color change is from yellow, through neutral gray, to a light purple color.

1 ml 0.02  $N$   $NaOH$  = 1.84 mg glycerol.

This procedure was applied to samples containing varying amounts of glycerol. A specially purified glycerol (99.55%), assayed by the periodate consumption method and specific gravity determination, was used to prepare a standard solution, from which aliquots containing known amounts of glycerol were taken. The results of the analyses are given in Table 1.

All recoveries in Table 1 are within  $100 \pm 0.9$  per cent.

The titration of formic acid with 0.02  $N$  sodium hydroxide using bromcresol purple indicator proceeds quite smoothly if the excess potassium periodate is destroyed. The iodates produced in the reduction have no

\* Excess periodate must be present. Test for periodate by adding  $NaHCO_3$  and  $KI$  to test portions; if excess is present, iodine will be liberated.



effect upon the titration. This was demonstrated by the following experiments:

A 0.1 gram sample of potassium iodate (approximately the amount formed by the reduction of the 22.7 ml of 0.02 *M* potassium periodate which was originally present in the 50 ml aliquot taken for titration) was dissolved in 30 ml of water and titrated with 0.02 *N* sodium hydroxide using 3 drops of the indicator. One drop (0.05 ml) of 0.02 *N* sodium hydroxide was sufficient to produce a sharp color change.

A solution of 22.7 ml of 0.02 *M* potassium periodate was diluted with 25

TABLE 1.—*Application of method to known amounts of glycerol*

SAMPLE	TITER FOR 50 ml ALiquot—ml OF 0.02 <i>N</i> NaOH	GLYCEROL FOUND	RECOVERY
mg. 40.29	9.95 9.91	mg. 40.28 40.12	per cent 100.0 99.6
30.22	7.47 7.46	30.24 30.20	100.1 99.9
20.14	4.99 4.96	20.20 20.08	100.3 99.7
10.07	2.51 2.51	10.16 10.16	100.9 100.9

ml of water, 10 drops of propylene glycol added, and the resulting solution allowed to stand for 10 minutes. Three drops of indicator were then added. Again 0.05 ml of 0.02 *N* sodium hydroxide was sufficient to titrate the acidity of the solution.

The above experiments indicate that no acidity is contributed by either the reduced potassium periodate or the oxidation products of the propylene glycol. On the other hand, the addition of as much as 2 ml of 0.02 *N* sodium hydroxide to 22.7 ml of 0.02 *M* potassium periodate containing bromocresol purple did not cause the indicator to change from a yellow to a purple color.

An oxidized glycerol solution was titrated to the selected light purple end point. The *pH* of the resulting solution when measured with a Beckman *pH* meter was found to be 6.4.

The method was then applied to a number of solutions containing known amounts of glycerol, propylene glycol, and ethylene glycol. The results are presented in Table 2.

All the recoveries in Table 2 are within  $100 \pm 0.3$  per cent.

The following test showed that neither the ethylene glycol or its oxidation products gave an acid blank:

Ten drops of ethylene glycol were diluted to 25 ml with water, 22.7 ml of 0.02 *M* potassium periodate added, and the solution allowed to stand for 10 minutes. Three drops of indicator were added and the solution titrated with 0.02 *N* sodium hydroxide. The titer was less than 0.05 ml of 0.02 *N* sodium hydroxide.

It has already been shown that the propylene glycol does not interfere with the procedure.

TABLE 2.—*Application of method to solutions containing known amounts of glycerol, propylene glycol, and ethylene glycol*

SAMPLE		TITER FOR 50 ML ALiquot—ML OF 0.02 <i>N</i> NaOH	GLYCEROL FOUND	RECOVERY
	mg		mg	per cent
Glycerol.....	20.14	4.99	20.20	100.3
Propylene glycol . . . .	33.16	4.97	20.12	99.9
Glycerol.....	20.14	4.98	20.16	100.1
Ethylene glycol.....	27.56	4.98	20.16	100.1
Glycerol.....	20.14	4.98	20.16	100.1
Propylene glycol.....	16.58	4.96	20.08	99.7
Ethylene glycol.....	13.78			
Propylene glycol.....	33.16	0.04	—	—
Ethylene glycol.....	27.56	0.04	—	—

### CONCLUSION

The alkali titration procedure of Shupe for the determination of glycerol has been modified. Excess periodate is reduced before the titration, and bromcresol purple is substituted for methyl red as the indicator.

By this method glycerol can be accurately determined alone, and in solutions containing propylene and ethylene glycols.

### RESIDUAL CHLORINE IN MILK AFTER THE ADDITION OF HYPOCHLORITE

By FERRIN B. MORELAND\* (Chemistry Department, Division of Public Health Laboratories, Kansas State Board of Health, Topeka, Kans.)

In interpreting the results of the Rupp test<sup>1</sup> on milk samples examined in the laboratory, the question arose as to the amount of residual free chlorine actually still present in the milks. A cursory examination of the

\* Present address: Biochemistry Department, Baylor University College of Medicine, Houston, Texas.

<sup>1</sup> U. S. Dept. Agr. Bull. 1114 (1922); A.O.A.C. *Methods of Analysis*, 6th ed. (1945), p. 317, 22.36–22.38, Hauser and King, *This Journal*, 28, 417–24 (1945).

## RESULTS OF RUPP TESTS

*Milk Kept at Room Temperature*

CL <sub>2</sub> ADDED (P.P.M.)	ELAPSED TIME	TESTS			
		a	b	c	d
1000	27 hrs.	Pale yellow	Yellow	Brown	Dark blue
	50 hrs.	—	Pale yel.	Brown	Dark blue
	5 days	—	—	Brown	Dark blue
500	27 hrs.	—	Pale yel.	Yel.-brn.	Dark blue
	50 hrs.	—	—	Yel.-brn.	Dark blue
	5 days	—	—	Yel.-brn.	Dark blue
200	27 hrs.	—	—	Yellow	Dark blue
	50 hrs.	—	—	Yellow	Dark blue
	5 days	—	—	Yellow	Dark blue
100	27 hrs.	—	—	Pale yel.	Dark blue
	50 hrs.	—	—	Pale yel.	Dark blue
	5 days	—	—	Pale yel.	Purple
	2 hrs.	—	Pale yel.	Yellow	Dark blue
	6 min.	Very pl. yel.	Pale yel.	Yellow	Dark blue
	18 hrs.	—	—	Yellow	Dark blue
	41 hrs.	—	—	Yellow	Dark blue
	4 days	—	—	Yellow	Dark blue
50	1½ hrs.	—	Pale yel.	Yellow	Dark blue
	18 hrs.	—	—	Yellow	Dark blue
	6 min.	—	Very pl. yel.	Yellow	Dark blue
	18 hrs.	—	—	Pale yel.	Purple
	41 hrs.	—	—	Pale yel.	Purple
	4 days	—	—	—	—
	10 min.	—	Pale yel.	Yellowish	Dark blue
	2 hrs.	—	—	Yellowish	Dark blue
10	19 hrs.	—	—	Yellowish	Dark blue
	6 min.	—	—	Very pl. yel.	Bluish
	18 hrs.	—	—	—	—
	41 hrs.	—	—	—	—
	4 days	—	—	—	—
5	6 min.	—	—	—	Pale purple
	18 hrs.	—	—	—	—
	41 hrs.	—	—	—	—
	4 days	—	—	—	—

*Milk Kept in Refrigerator*

Cl <sub>2</sub> ADDED (P.P.M.)	ELAPSED TIME	TESTS			
		a	b	c	d
100	12 min.	Pale yellow	Pale yel.	Yellow	Dark blue
	1 hr.	Pale yellow	Pale yel.	Yellow	Dark blue
	2 hrs.	—	Pale yel.	Yellow	Dark blue
	6 hrs.	—	Very pl. yel.	Yellow	Dark blue
	1 day	—	—	Yellow	Dark blue
	2 days	—	—	Yellow	Dark blue
	3 days	—	—	Yellow	Dark blue
	5 days	—	—	Yellow	Dark blue
	10 days	—	—	Yellow	Dark blue
	$\frac{1}{2}$ hr.	Pale yellow	Pale yel.	Yellow	Dark blue
	21 hrs.	—	—	Yellow	Dark blue
	$\frac{1}{2}$ hr.	—	—	Yellowish	Dark blue
	21 hrs.	—	—	Yellowish	Dark blue
	3 days	—	—	Yellowish	Dark blue
20	1 hr.	—	—	Yellowish	Bl.-purple
	21 hrs.	—	—	Yellowish	Dark blue
	3 days	—	—	Yellowish	Dark blue
5	1 hr.	—	—	Very pl. yel.	Purple
	21 hrs.	—	—	—	—
	3 days	—	—	—	—

literature did not shed any light on the question, and the experiments herein described were therefore undertaken.

Random samples of raw market milk were used. The chlorine was added in the form of a commercial bleaching solution (Purex) containing, at the time of use, enough sodium hypochlorite to yield about 1.5 per cent of available chlorine. The Rupp test was performed as described in the A.O.A.C. Methods of Analysis. Residual chlorine was determined by titrating a 25 ml sample of the milk with 0.01 *N* sodium thiosulfate after the addition of 5 ml of 7% potassium iodide solution, in the presence of starch added near the end point.

The residual chlorine of the milk decreased rapidly as shown in the tables. At a chlorine dosage calculated to produce 100 p.p.m. in the milk, the value dropped to zero in around a quarter of an hour. The response to tests *a* and *b* decreased correspondingly; however, tests *c* and *d*, at this dosage, continued positive long after the residual chlorine, as indicated by the thiosulfate titration, reached zero. There was some falling off after several days at 50 p.p.m., and in a few hours at lower concentrations.

The term "available chlorine" as used in the heading of the table inter-

## TITRATION OF FREE CHLORINE

<i>Milk Kept at Room Temperature</i>			<i>Milk Kept in Refrigerator</i>		
Cl <sub>2</sub> ADDED (P.P.M.)	ELAPSED TIME	Cl <sub>2</sub> FOUND (P.P.M.)	Cl <sub>2</sub> ADDED (P.P.M.)	ELAPSED TIME	Cl <sub>2</sub> FOUND (P.P.M.)
1000	31 min.	244	100	1 min.	14
	2½ hrs.	167		6 min.	17
	26 hrs.	7		11 min.	7
500	22 min.	<150		15 min.	4
	2½ hrs.	87		20 min.	3
	26 hrs.	0		30 min.	1
200	12 min.	41		60 min.	1
	2½ hrs.	14	50	2 min.	17
	26 hrs.	0		4 min.	15
100	7 min.	17		6 min.	14
	49 min.	0		10 min.	11
	5 min.	<24		21 min.	8
	2 hr.	0		30 min.	8
	1 min.	14		60 min.	3
	1 hr.	0		123 min.	0
	5 min.	<10	20	2 min.	0
	15 min.	0		1 min.	0
50	5 min.	0	5	2 min.	0
	6 min.	0			
10	6 min.	0			
5	6 min.	0			

Note: Horizontal lines set off separate experiments.

preting the reactions to the Rupp test (*Methods of Analysis*, 22.38) is apt to be misleading since the values given are for the amount of available chlorine which is present at the instant of its addition to the milk, rather than when the Rupp test is actually performed, perhaps much later.

## A CRITICAL STUDY OF THE MODIFIED AYRE-ANDERSON METHOD FOR THE DETERMINATION OF PROTEOLYTIC ACTIVITY<sup>1</sup>

By BYRON S. MILLER (Federal Hard Wheat Quality Laboratory, Manhattan, Kansas)

The determination of proteolytic activity of flour and flour products is somewhat difficult because of the small amount of proteolytic enzymes present in these materials. A survey of the literature indicates that of all proposed methods the one by Ayre and Anderson (1), as modified by Landis (5) and Redfern (10), is the simplest and least troublesome. This procedure is based on the semi-autolytic digestion of the sample in the presence of an auxiliary substrate (Bacto-hemoglobin) at controlled temperature and pH. The hemoglobin provides additional readily attackable protein material to supplement that present in the flour. The undigested protein is precipitated with trichloroacetic acid after an initial short time as well as after a final long digestion period, and the unprecipitated non-protein nitrogen is determined by a Kjeldahl procedure. The increase in non-protein nitrogen determined from the two digestions is a measure of the proteolytic activity of the sample.

Hildebrand (3) compared the Ayre-Anderson precipitation method with the Landis and Frey rate-of-gelation method (6) and found that both methods gave essentially similar results and had approximately the same experimental error and ability to differentiate between samples. The modified Ayre-Anderson method has been subjected to a collaborative study, and on the basis of this study Redfern (11) recommended that the semi-autolytic method be discontinued. The data from different laboratories and those from the same laboratory obtained on different days showed poor agreement. The present investigation deals with the conditions necessary to provide a reproducible and convenient method of determining proteolytic activity based on a modified Ayre-Anderson procedure.

### METHODS

**Digestion Procedure:** A 5-g sample of flour is weighed into a 125 ml Erlenmeyer flask and to this is added 0.625 g (moisture-free basis) of Bacto-hemoglobin.<sup>2</sup> The mixture is agitated by rotating the flask until the flour and substrate are intimately mixed. This procedure facilitates complete suspension of the hemoglobin and flour. A volume of 25 ml of at-tempered 0.1 M sodium acetate-0.1 M acetic acid buffer solution at pH 4.7 is added. After agitation and stirring with a glass rod until a uniform

<sup>1</sup> Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Department of Milling Industry, Kansas State College. Published as Contribution No. 137, Department of Milling Industry, Kansas State College.

<sup>2</sup> The hemoglobin used in these studies was obtained from the Difco Laboratories, Detroit, Michigan.

suspension is obtained, the flask is tightly stoppered and placed in an automatic shaking device fitted in a constant temperature water bath held at  $40.0 \pm 0.1^\circ\text{C}$ . The automatic device is not absolutely essential, however, since manual agitation of the digestion flasks each hour gave results identical to those obtained by employing continuous agitation.

A separate flask corresponding to a single proteolytic determination is used for each point of activity. After a definite reaction time five ml of 36% trichloroacetic acid is added to each flask and shaken in the bath for an additional five minutes. The contents of each flask are then poured into a centrifuge tube and centrifuged for five minutes at 1,800 r.p.m. The centrifugate is filtered through filter paper and the solid residue is discarded. A 5-ml portion of this solution is pipetted directly into a Kjeldahl flask and analyzed for nitrogen. A precipitation is made on each sample after 15 minutes of digestion, which takes the place of a blank determination. This time is sufficiently long to allow the original soluble constituents of the flour time to dissolve completely. The final digestion period is for five hours.

*Kjeldahl Procedure:* The standard Kjeldahl-Gunning method (7) is used; however, a digestion time of 20 minutes is sufficient. Each still is checked for leaks and duplicability by digesting aliquots of ammonium oxalate solution. A definite volume of water (350 ml) is used to dilute the acid and is added in such a way as to wash down all of the trichloroacetic acid which has condensed in the neck of the flask during the digestion process. The concentrated alkali is also added in such a manner as to lave the neck of the flask and thus neutralize any portions of acid remaining on the neck of the flask. The unneutralized standard acid is back-titrated with 0.0714 *N* sodium hydroxide.

*Calculation of Activity:* Proteolytic activity is calculated on the basis of milligrams of non-protein nitrogen released by the enzymes in 10 g of flour. The back-titration value for the five-hour digestion is corrected by subtracting from it the back titration volume for the 15 minute digestion. This difference represents the arbitrary proteolytic activity and is translated into mg *N* produced from the 5-g sample. This value is further corrected to show the non-protein nitrogen released from a 10-g sample of flour on a 14 per cent moisture basis.

## RESULTS

*The Use of Various Concentrations of Hemoglobin.*—Bacto-hemoglobin of the "Difco" brand used for this work is prepared from defibrinated beef blood by washing the cells with a saline solution prior to desiccation. Other commercial hemoglobins are prepared from unwashed cells or from whole blood and are not suitable for this work. Samples from different lots of "Difco" hemoglobin vary only slightly in their availability to proteolytic action, as is indicated by the data in Table 1. The standard devia-

TABLE 1.—*Replicability of proteolytic activity determinations using different lots of Bacto-hemoglobin*

FLOUR	HEMOGLOBIN	PROTEOLYTIC ACTIVITY	STANDARD DEVIATION	MEAN
	Lot no.	Mg nitrogen/10 g flour		
Commercial patent	A	18.2	0.7	18.7
" "	B	18.7		
" "	C	18.7		
" "	C	18.2		
" "	D	19.9		
Malted wheat flour	A	50.9	0.5	50.5
" " "	B	50.3		
" " "	C	50.3		
" " "	C	49.7		
" " "	D	50.9		

tion is presented for both flours. Each sample was weighed on a moisture-free basis and treated like all the others in every respect.

The quantity of the auxiliary substrate influences the extent of proteolytic action up to a certain limit. The minimum amount on the moisture-free basis was determined to be 2.5 per cent. As will be seen in Figure 1, further additions of hemoglobin did not increase the non-protein nitrogen released.

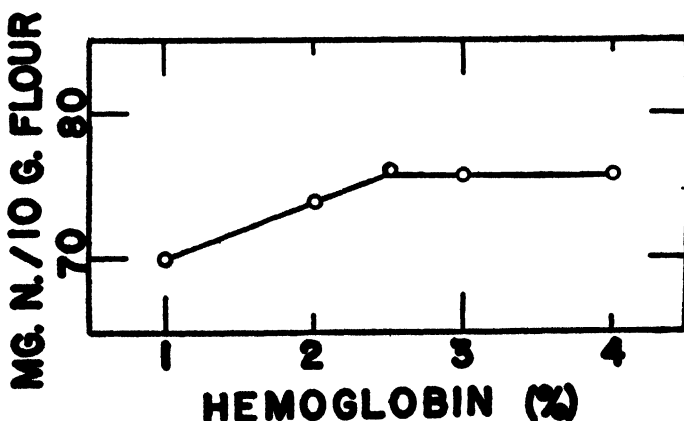


FIG. 1.—Proteolytic activity of malted wheat flour as influenced by the amount of Bacto-hemoglobin present.

**Digestion Temperature.**—The temperature of digestion has a pronounced effect on the amount of soluble nitrogen containing compounds produced. Previous workers have generally used temperatures from 37° to 45°C in determining proteolytic activity. The data in Figure 2 indicates that the greatest activity in wheat flour is attained at approximately



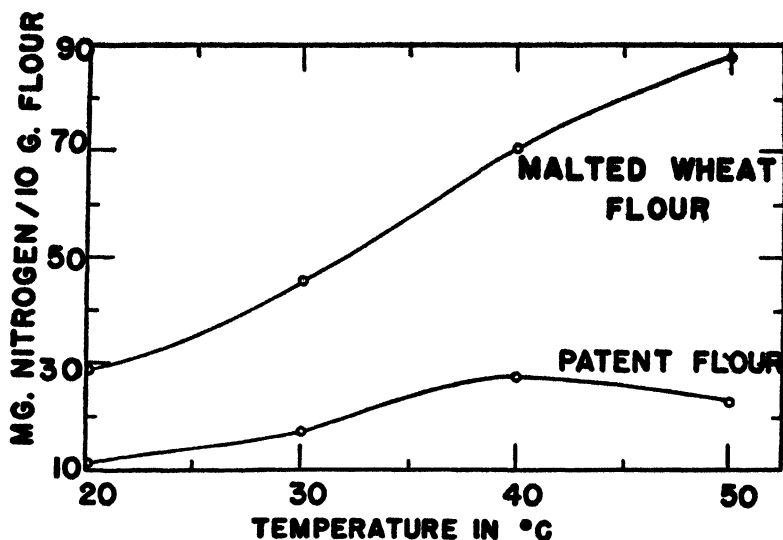


FIG. 2.—Proteolytic activity as influenced by digestion temperature.

40°C, but malted wheat flour appears to have an optimum beyond 50°C. A temperature of 40°C was used in this investigation for the standardized procedure.

*Digestion Time.*—The amount of proteolysis was determined for malted wheat flour and a commercial patent flour over periods varying from 5 minutes to 8 hours. These results are presented in Figures 3 and 4. No particular advantage is gained by allowing the reaction to progress beyond five hours; furthermore, this time period fits well into an experimental day.

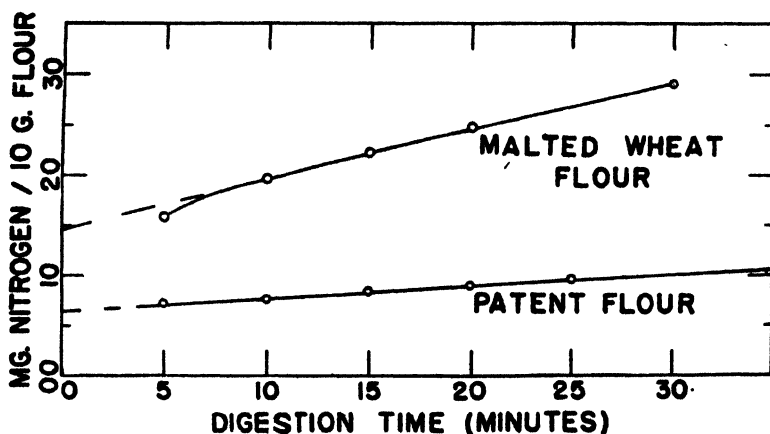


FIG. 3.—Proteolytic activity during the initial period of digestion.

The time required for the initial soluble unprecipitable nitrogen products to go into solution was arbitrarily selected from Figure 3 as fifteen minutes. The blank determination is then the titration value obtained from this 15 minute digestion and the empirical measure of proteolytic action is the difference between the value for the five hour digestion and the 15 minute digestion.

*Influence of pH.*—The effect of  $pH$  on the proteolytic activity of both

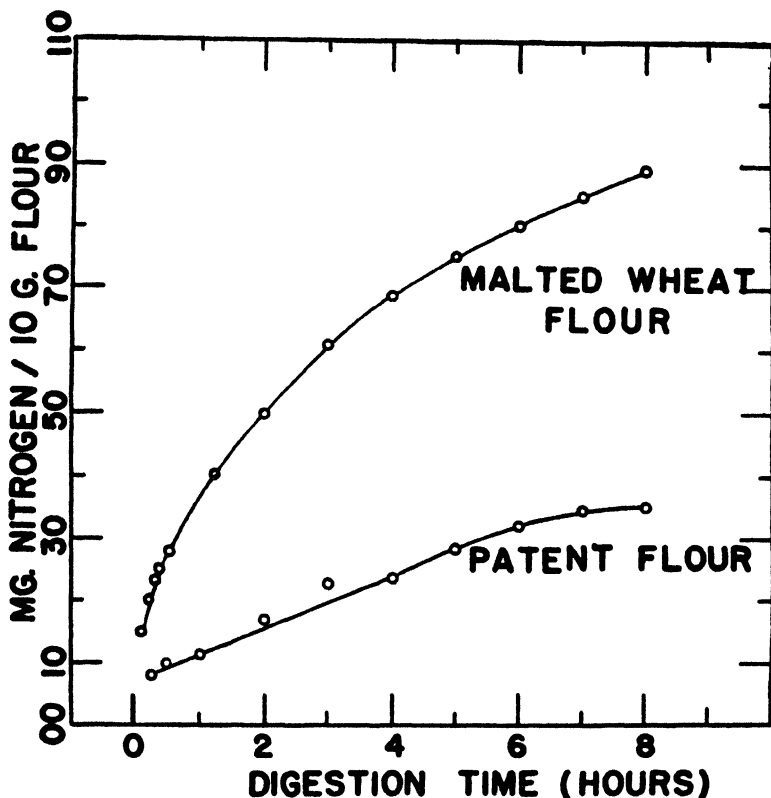


FIG. 4.—Proteolytic activity as influenced by long-time digestion.

malted wheat flour and commercial flour was determined by using a five-hour digestion period at 40°C with 2.5 per cent hemoglobin. The various  $pH$  values were maintained by using variations of the 0.1  $M$  sodium acetate-acetic acid buffer. The  $pH$  readings were checked at the beginning and end of each digestion; no changes in  $pH$  were observed. In the case of both flours the optimum  $pH$  under the conditions studied was approximately 3.5. This low optimum value does not appear to be due to acid denaturation of the substrate, since an aliquot of hemoglobin treated with

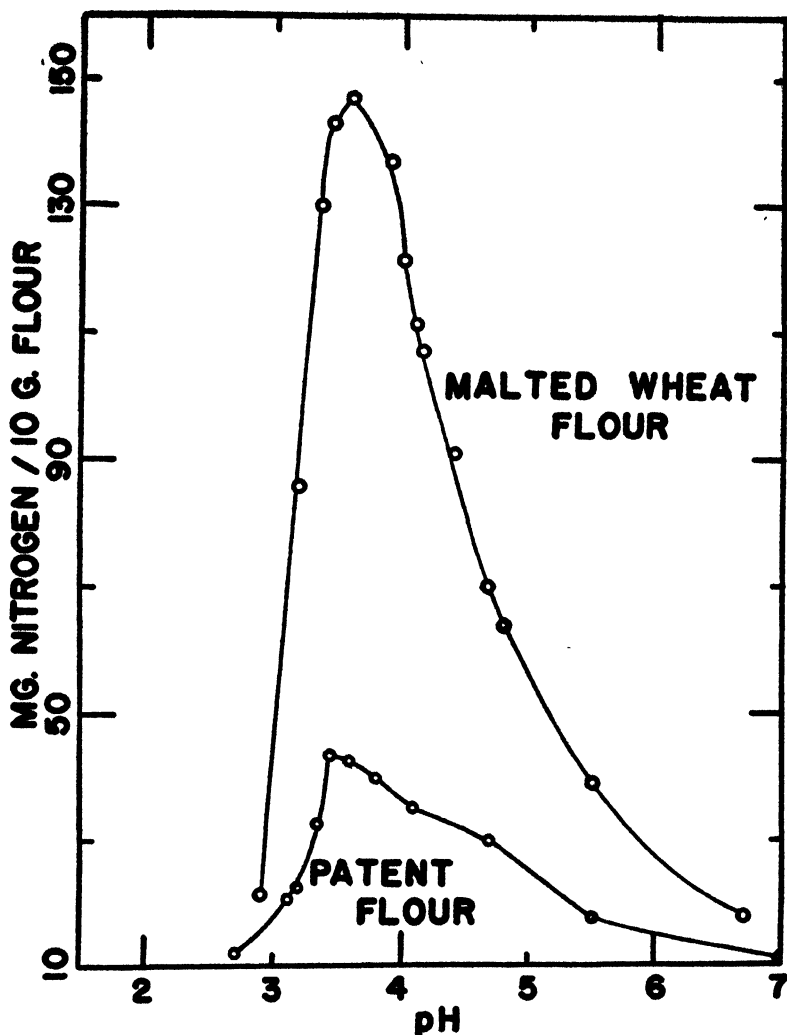


FIG. 5.—Proteolytic activity as influenced by pH.

strong acid and later adjusted to a pH of 4.7 and used as a substrate gave a duplicate result to an aliquot adjusted to a pH of 4.7 without acid treatment. The results of the pH study are shown in Figure 5.

Buffer solutions of different composition yield decidedly different results even at the same pH values, as indicated in Table 2. This may be due, as is indicated by previous workers, to an inhibitory effect of certain ions. The present work as well as data by other workers indicates that both the chloride and citrate ions have a detrimental effect on proteolytic activity.

*Influence of Trichloroacetic Acid Concentration.*—The concentration of

TABLE 2.—*Effect of different buffers on the proteolytic activity during a five-hour digestion period at 40°C*

BUFFER	pH	FLOUR	PROTEOLYTIC ACTIVITY
			<i>Mg nitrogen 10 g flour<sup>a</sup></i>
0.1 M NaAc-0.1 M HAc	4.7	Over-malted wheat flour	183.6
0.12 M NaAc-0.08 M HAc=0.2 M NaCl	4.8	Over-malted wheat flour	154.8
0.24 M NaAc-0.16 M HAc=0.4 NaCl	4.8	Over-malted wheat flour	130.8
0.25 M Na Cit-0.25 M HCl	4.8	Over-malted wheat flour	129.6
0.1 M NaAc-0.1 M HAc	4.7	Malted wheat flour	76.2
0.2 M NaAc-0.2 M HAc	4.7	Malted wheat flour	76.2

\* These values are for the five-hour digestion and are not corrected for the blank.

trichloroacetic acid used as the precipitating agent for the undigested protein nitrogen plays a distinct role in the results obtained. As is indicated by Figure 6, a minimum of 6 per cent by weight of acid is necessary to precipitate the maximum amount of protein nitrogen present.

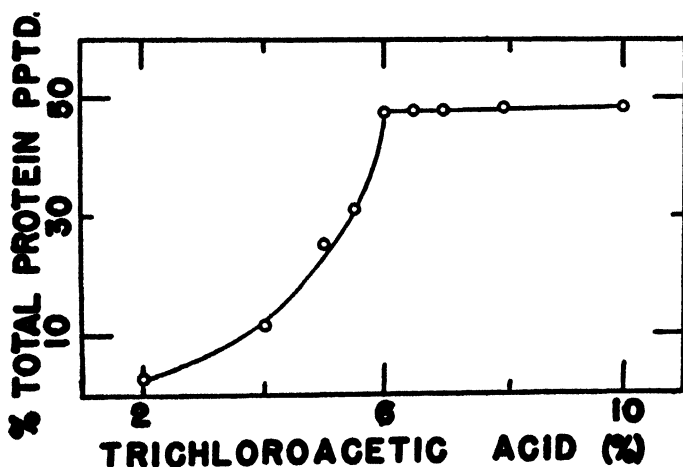


FIG. 6.—The relationship of the amount of total protein precipitated to the per cent (by weight) of trichloroacetic acid used.

**Replication of Results.**—Results obtained on a commercial flour and on a malt flour are tabulated in Table 3. Each result was obtained on a different day. The standard deviation is shown for each flour.

TABLE 3.—*Replicability of results obtained on separate days using the same experimental conditions*

FLOUR	TRIAL	PROTEOLYTIC ACTIVITY	STANDARD DEVIATION	MEAN
		<i>Mg nitrogen/10 g flour</i>		
Commercial patent	1	18.7	0.65	19.0
" "	2	20.4		
" "	3	18.2		
" "	4	19.3		
" "	5	19.3		
" "	6	18.7		
" "	7	19.3		
" "	8	18.6		
" "	9	19.5		
Malted wheat flour	1	50.3	0.47	49.8
" " "	2	50.3		
" " "	3	49.2		
" " "	4	49.7		
" " "	5	49.7		

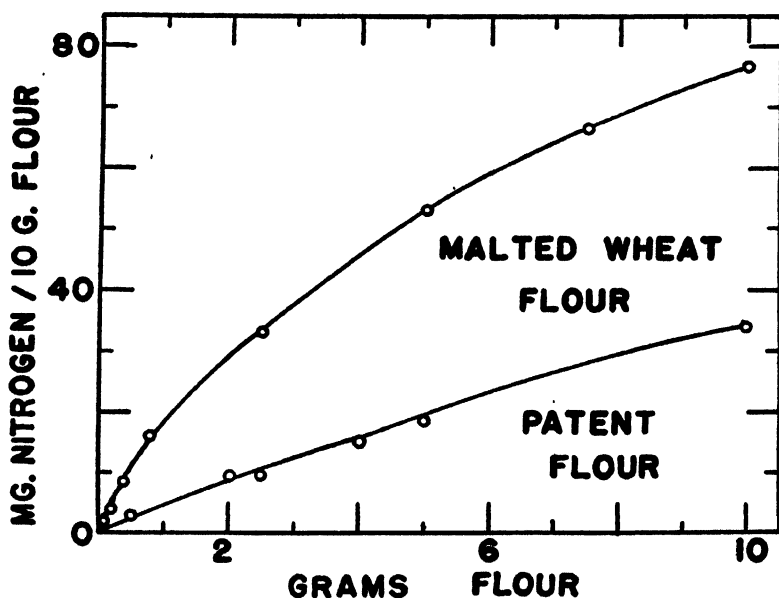


FIG. 7.—Proteolytic activity as influenced by enzyme concentration.

*Proteolytic Activity as Influenced by Enzyme Concentration.*—Figure 7 shows the relation of the amount of enzyme present to the amount of soluble nitrogen liberated during the usual five-hour digestion period. Since an excess of hemoglobin substrate is present, the addition of various amounts of flour with proportionate variation in protein content is of no consequence.

#### DISCUSSION

It is hoped that the studies reported here will lend confidence to the modified Ayre-Anderson method of determining proteolytic activity. An effort has been made to adapt the method to levels of activity varying from that of commercial bread flour to that of malted wheat flour.

Bacto-hemoglobin, unlike casein and gelatin, is a reproducible substrate. Different lots of hemoglobin are digested at the same rate by a given proteinase solution. Even when peptidase is present in addition to proteinase, Anson (2) states that the formation of products not precipitable by trichloroacetic acid is due as far as is known to proteinase action alone.

Previous workers (4, 8, 9) have found that the *pH* optimum for the proteolytic enzyme system in flour and wheat germ varied with the different proteins used as substrates. Howe and Glick (4) found the *pH* optimum to be 5.0 for the enzymes in whole wheat, germ, clear, and patent flours, with and without the addition of casein as an auxiliary substrate. In papers dealing with the proteolytic enzymes of sprouted wheat, Mounfield (8, 9) observed a *pH* optimum of 4.1 when edestin was used as an auxiliary substrate, 5.1 when gelatin was used, and 6.0 when an acetic acid dispersion of gluten was added. The present work indicates an optimum *pH* of 3.5 when Bacto-hemoglobin is used as the auxiliary substrate. Considering this variability of *pH* optimum and the possibility of hemoglobin denaturation at a *pH* of 3.5, it was decided to employ an arbitrary *pH* of 4.7 in the present investigation. This *pH* is within the normal operating range of enzymes in the natural state and can be maintained by a sodium acetate-acetic acid buffer system which has non-inhibitory ions. The buffer system proposed by Landis (5) contained chloride ions which are shown to be inhibitory to proteolytic action.

The greatest proteolytic activity occurred at a temperature of 40°C for patent flour, while a temperature of above 50°C provided the maximum activity for malted wheat flour. A standard arbitrary temperature of 40°C was adopted for this study as was suggested by Landis (5); other temperatures are, however, equally adaptable.

Relatively few operational changes in the method as modified by Landis (5) and Redfern (10) were made. The dry hemoglobin is added to individual reaction flasks instead of being added as a suspension of hemoglobin and buffer, in order to insure a more uniform and complete suspension of flour and substrate. A 125 ml Erlenmeyer flask was more adaptable to the reaction volume. The blank digestion time has been arbitrarily reduced from 25 minutes to 15 minutes on the basis of the curves in Figure 3.

In view of past criticism of this method it is necessary to stress certain precautions which this study has shown to be necessary in order to obtain satisfactory results. Most, if not all, of the difficulty in replication of results within a single laboratory and between collaborators is possibly due to differences in carrying out the Kjeldahl procedure. Prior to distillation of the digested mixture it is essential that all of the trichloroacetic acid which has volatilized and deposited on the neck of the flask during the digestion period be washed down into the solution where it can be neutralized by the sodium hydroxide. Even then a small portion of the trichloroacetic acid distills over into the receiver acid. This is compensated for, however, by subtracting the value for the 15-minute digestion from that for the five hour digestion since the same quantity of trichloroacetic acid is evolved in both cases.

Each Kjeldahl still should be carefully checked for leaks by distillation of a known quantity of nitrogen containing salt. Only those stills giving results which agree with the theoretical values should be used.

A consideration of Figure 7 indicates that the proteolytic activity for malted wheat flour is not directly proportional to the quantity of enzyme used in the experimental determination. Further, Figure 4 indicates that the proteolytic activity for this type of flour is not linearly related to the time used for digestion. The enzyme-substrate reaction is thus not a zero order reaction, in the case of malted wheat flour, and precludes the determination of specific proteolytic activity independent of time and quantity of enzyme. Similar curves are shown by Anson (2) for pepsin, trypsin, papain, and cathepsin. Figures 4 and 7 indicate essentially a zero order reaction for ordinary patent flour under the conditions of this experiment.

Relative proteolytic activities, however, are readily obtainable provided the conditions of the experiment are rigidly defined. Various patent flours produce curves which parallel closely the one shown in Figure 7. Thus relative proteolytic activity of a series of flours may be determined by comparing the mg of soluble nitrogen produced under specified conditions by a given quantity of these flours.

By using smaller quantities of substances having extremely high proteolytic activity, values may be determined for such substances as bran or germ fractions. Likewise, an accurate measurement of the activity of purified proteolytic enzymes is possible. In all cases it is necessary that the quantity of proteolytic enzymes is such that the amount of Bacto-hemoglobin present is not a limiting factor in the results obtained. For example, it is necessary to use no more than 25 mg of mold bran with the ordinary 2.5 per cent hemoglobin suspension in evaluating the proteolytic activity of mold bran preparations. A curve similar to that shown in Figure 1 should be obtained prior to evaluating the proteolytic activity of any substances other than flour or malted wheat flour.

It is frequently convenient, especially when the proteolytic activity is high, to use an extract of the material being analyzed rather than a quan-

tity of the solid material itself. In order to get complete suspension of the hemoglobin in such cases it is necessary to add approximately three grams of finely divided pumice or sand to the dry hemoglobin prior to the addition of liquid. In this case a more concentrated solution of trichloroacetic acid must be added at the end of the digestive period in order to limit the volume to 30 ml.

#### SUMMARY

The modified Ayre-Anderson method for the determination of proteolytic activity has been critically studied to determine the effects of various conditions on the results obtained. A patent flour and a malted wheat flour have been investigated.

A concentration of 2.5 per cent (dry weight basis) Bacto-hemoglobin was shown to be sufficient for levels of proteolytic activity as high as those found in malted wheat flour.

The activity of patent flour proteinase reached a maximum at 40°C. For malted wheat flour the optimum temperature was above 50°C.

The optimum pH for digestion was shown to be approximately 3.5 for both patent flour and malted wheat flour when using Bacto-hemoglobin as an additional substrate.

The use of at least 6 per cent (by weight) trichloroacetic acid as a precipitating agent of proteins is required, and certain precautions necessary in the Kjeldahl determinations of the soluble nitrogen components are indicated.

The level of precision obtained with substances having high or very low proteolytic activity appears to be satisfactory if the conditions herein described are maintained.

The method is adaptable to the determination of proteolytic activity in bran and germ fractions or purified proteolytic enzymes.

#### ACKNOWLEDGMENT

The author wishes to acknowledge the assistance given by other members of the Department of Milling Industry in the preparation of this paper and to Dean F. Willibey who did a large portion of the experimental work.

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## A MICROCHEMICAL SPOT TEST FOR MAMMALIAN URINE CONTAMINATION ON FABRICS

By N. H. ISHLER, KATHERINE SLOMAN, and MARY E. WALKER (General Foods Corporation, Central Research Laboratories, 11th & Hudson Streets, Hoboken, New Jersey)

Before the 6th edition of the A.O.A.C. Methods was received, this laboratory was requested to recommend a satisfactory method of confirming the evidence of fluorescence on flour bags by a microchemical type of test which should be specific for urine.

Attempts were made to develop a spot test type of reaction for well known constituents of urine, such as creatinine and urea. Direct chemical methods such as the familiar Biuret test for urea and the Weyl nitroprusside test (1), for creatinine, as applied directly to fabric, were unsuccessful. Evolution of ammonia from urea by urease and its detection by means of a sensitive manganese nitrate-silver nitrate impregnated paper (2) gave very satisfactory results and provided a rapid and sensitive test for urea. Upon receipt of the 6th edition of the A.O.A.C. Methods (3), it was considered desirable to make a comparison between the published methods and that developed in this laboratory as to dependability, sensitivity, and ease of manipulation. The new method is described in detail, followed by comparison with the A.O.A.C. procedures.

### EXPERIMENTAL

#### REAGENTS

*Urease Soln.*—A 10% slurry of Jack Bean Meal in water is prepared and allowed to settle a few minutes; the supernatant liquid is used as urease soln. This slurry may be stored for as long as a week in a refrigerator without apparent loss of effectiveness.

*Manganese Nitrate-Silver Nitrate Soln (2).*—14 ml of 50% soln of  $\text{Mn}(\text{NO}_3)_2$  are made up to 100 ml; add this soln to a soln of 4.0 g of  $\text{AgNO}_3$  made up to 100 ml. Neutralize the combined solns with 0.1 N NaOH until a black precipitate first forms. Filter, and store filtrate in dark reagent bottle.

*Filter Paper Impregnated with Manganese-Silver Soln.*—Coarse filter paper is soaked in the neutralized  $\text{Mn}(\text{NO}_3)_2$ - $\text{AgNO}_3$  soln and dried quickly on the metal surface of a steam bath heated to approximately 100°C. (Samples of this paper stored for one week in a clean bottle in the dark developed brown stains. These did not interfere with the operation of the test. However, it is considered desirable to prepare a fresh supply, rather than to use paper stored longer than one week).

#### METHOD

Prior to applying the spot test, outline with a pencil on the fabric, a stain suspected of being due to urine, while observing its fluorescence under ultra-violet irradiation. If the stain is clearly visible under ordinary illumination, this step will not be necessary. Apply two to four drops of urease soln to the stained area of the cloth and allow to soak in for 5–10 seconds. Then place the cloth on the surface of a heated steam bath, and immediately place over it a piece of impregnated paper so that the paper is wetted by the damp area of the cloth. Maintain a firm contact by laying a piece of glass stirring rod across the moistened area and pressing firmly. If the stain was due to urine, a black spot appears on the filter paper in about thirty seconds.

Ammonia vapor in the laboratory should be avoided during the running of the test. The color development characteristic of ammonia is sufficiently rapid so that there is little likelihood of a false indication from this source. However, a sensitized paper placed beside the specimen under test serves to indicate the possibility of error.

Some heavily sized fabrics may cause difficulty with this test because of the inability of the urease solution to wet and penetrate the fabric. It has been determined that 0.1 per cent of a wetting agent such as Nacconol (National Aniline) added to the urease solution to aid penetration will neither give a false positive reaction in the absence of urine nor prevent the usual color development when urea is present.

Experiments showed that the procedure described above still gave a positive indication of the presence of urine on cloth powdered with grain products such as oatmeal, corn meal, fine wheat flour and "all purpose" flour. These grain products did not give a false positive reaction when urine was not present.

Sulfides ( $K_2S$  was used) can produce a black stain when direct contact with the paper is maintained. Sucrose and dextrose do not interfere, but molasses contains reducing substances which give a false positive reaction.

In the presence of either sulfides or strongly reducing organic materials, a false indication may be avoided by supporting the impregnated paper above the sample on two horizontal stirring rods placed an inch or so apart on the cloth. The test may be performed entirely satisfactorily in this manner, but the time required for development of a black stain due to urine is approximately doubled. Wetting the sensitized paper retards the test still further. No substance likely to be encountered in fabrics has been found to prevent a positive reaction when urine was present.

#### COMPARISON OF METHODS

The A.O.A.C. Methods were followed in order to form an estimate as to relative advantages of any one method.

The "Urease Test for Urea" (42.98, A.O.A.C., 6th Ed.) depends on the evolution of ammonia from urea by the action of urease as does the spot test here described. The ammonia is then detected by the formation of  $(NH_4)_2PtCl_6$ . This test was also found to be valid in the presence of grain materials likely to be encountered in flour sacking.

The "Xanthydrol Test for Urea" (42.99, A.O.A.C., 6th Ed.) depends on the formation, and microscopic examination, of xanthydrol urea. We used Eastman Xanthydrol #1559 for this test, and obtained a positive reaction, as evidenced by the characteristic star shaped crystals, even when urea was not present. No attempt was made to further purify the xanthydrol to determine whether the false positive test could be eliminated.

The method "Extraction of Urea and Crystallization of Urea Nitrate"

TABLE 1.—*Comparison of methods*

TEST	A.O.A.C. REF. 6TH ED. (1945)	MINIMUM CONC. OF UREA TO WHICH TEST IS POSITIVE	TIME REQUIRED FOR POSITIVE TEST AT MINIMUM CONCENTRATION
Urease Test for Urea	<sup>sec</sup> 42.98	<sup>per cent</sup> 0.05	<sup>min</sup> 12
Xanthydrol Test for Urea	42.99	Gives positive reaction with clean cloth	—
Extraction of Urea and Crystallization of Urea Nitrate	42.100	Characteristic urea crystals but not any urea nitrate crystals	—
Microchemical Spot Test	—	0.01	0.5

(42.100, A.O.A.C., 6th Ed.) was found to be satisfactory, producing characteristic needle shaped crystals of urea by extraction with alcohol and acetone. However, it was found difficult to convert these crystals to a recognizable form of urea nitrate as described in the A.O.A.C. procedure.

Aqueous solutions of 1.0, 0.1, 0.05, 0.025, 0.01, and 0.005% urea were prepared. Samples of fabric were spotted with four drops of each the urea solutions. After drying, the spotted areas were tested by the A.O.A.C. procedures as well as by the proposed spot test. The results are recorded in Table 1. The time required by the new method for a positive reaction (and hence the likelihood of extraneous contamination) is less, and the sensitivity to small amounts of urea is greater, under the conditions of this test, than in the "Urease Test for Urea."

#### SUMMARY

A new spot test is described for the identification of urine stains on fabrics such as flour sacking. The method is rapid, simple, and dependable. Unusually low concentrations of urea produce a definite positive reaction. Excessive ammonia fumes in the laboratory must be avoided since the test depends on the evolution of ammonia by the action of urease on urea present as a constituent of the urine stain. The ammonia is detected by the black stain produced by its reaction with manganese-silver impregnated paper in close contact with the test specimen.

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## ASCORBIC ACID IN GRAPEFRUIT JUICE, ORANGE JUICE, AND THEIR BLENDS: 1943

By J. W. SALE, *et al.*\* (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

In 1943 the Food and Drug Administration determined ascorbic acid retention during manufacturing and storage, in grapefruit juice, orange juice, and their blends, following the same general procedure used for tomatoes and tomato juice(1). Volatile oil, suspended matter, acidity, pH, soluble solids, consistency, and other ingredients or properties were also determined in the canned juices. Since that work was done, other investigators (2), (3), (4) have reported their findings on work conducted along similar lines.

Ascorbic acid retention during manufacturing was ascertained by collecting samples of juices in the factories at various stages of manufacture, taking them to the titrating table in the factory, and immediately determining their ascorbic acid content. Usually, during a day's visit to a factory, three series of four samples each were collected at the following sampling places: (1) after extraction; (2) after screening; (3) after pasteurization; (4) after closing can. Also, some samples were collected and titrated after deaeration. After the completion of a series, enough additional sealed cans of the juices were collected in succession from the production line to furnish samples for later chemical analysis including the determination of ascorbic acid retention. Retention was ascertained by determining ascorbic acid, usually in three duplicate cans, after each period of storage (3, 6, 9, and 12 months) at room temperatures, in Atlanta, in Houston, and in Los Angeles.

Ascorbic acid was determined by the 2,6-dichlorophenolindophenol titration method described by Hall (5). The samples were filtered through well-washed cheesecloth, discarding first portion of filtrate. Two portions, of 4 or 5 ml each, of the juices were pipetted into an equal volume of the metaphosphoric-acetic acid reagent and each portion was rapidly titrated with the dye. The dye was standardized daily with the purest ascorbic acid obtainable, usually Eastman catalogue No. 4640. Ascorbic acid purity was determined by titration with iodine standardized against National Bureau of Standards  $As_2O_3$ .

Table 1 deals with the scope of the work, and Tables 2, 3, and 4 contain such of the data as appear necessary to establish the range of factory and storage losses of the various citrus juices investigated. A flow diagram of

\* The work was directed and participated in by the author of record, assisted by Dr. R. A. Osborn of the Food Division (W. B. White, Chief); and the following chemists and inspectors made very material contributions:

Armstrong, J. F.  
Badger, C. H.  
Born, R. B.  
Durrenberger, W. R.  
Fowler, G. R.

Harris, M.  
Holliday, D. J.  
Koelsche, C. L.  
Lint, H. L.  
Moses, W. R.

Rents, M. O.  
Rynerson, G. W.  
Sanders, J. W.  
Wilson, J. B.  
Winkler, W. O.

the manufacture of orange juice and grapefruit juice has been published (6), and might well be consulted in connection with this report.

Data on factory equipment and manufacturing processes were recorded but, because of the confidential nature of much of that information, are not here included. Source and variety of fruit were noted. The basic data

TABLE 1.—*Scope of investigation in 1943*  
(Exclusive of data in Tables 5 and 6)

CITRUS AREAS	NO. OF FACTORIES VISITED	NO. OF SERIES <sup>1</sup>	NO. OF SAMPLES	NO. OF DETERMINATIONS IN FACTORY	NO. OF DETERMINATIONS ON STORED SAMPLES
<i>Sweetened grapefruit juice</i>					
Florida	3	4	18	53	48
<i>Unsweetened grapefruit juice</i>					
Florida	10	26	120	243	120
Texas	10	29	119	184	168
Calif., Ariz.	4	13	42	42	27
<i>Sweetened orange juice</i>					
Florida	5	14	62	130	72
<i>Unsweetened orange juice</i>					
Florida	3	7	31	68	36
Texas	1	3	11	16	16
Calif., Ariz.	8	23	83	83	65
<i>Sweetened blends</i>					
Florida	3	7	33	93	60
Texas	1	3	10	13	19
Totals	48 <sup>2</sup>	129	529	925	631

<sup>1</sup> A series consisted of from 3 to 5 (usually 4) individual samples taken at different steps in the process of manufacture of the juice. Usually 3 series were collected at each factory and assigned one number.

<sup>2</sup> 41 separate factories: Florida 18; Texas 10; Calif., Ariz. 13.

and compilations not included, as well as the factory data of a non-confidential nature, can be consulted in the files of the Food Division of the Food and Drug Administration.

The screened juices, instead of the extracted juices, are compared with the canned juices in Tables 2, 3, and 4, because the ascorbic acid was not determined in the freshly extracted juices in the samples of 49 of the series. However in 80 of the series, where ascorbic acid was determined in both the freshly extracted juices and the screened juices, the average of ascorbic acid in all of the freshly extracted juices was 45.2, and that in all of the screened juices was 45.1.

Apparent gains in ascorbic acid content during factory processing are

TABLE 2.—Retention of ascorbic acid in canned grapefruit juice (27 factories)

DATE 1945	CODE NO. OF FAC- TORY	ASCORBIC ACID FOUND IN SCREENED JUICE				PER CENT ASCORBIC ACID RETAINED IN CANNED GRAPEFRUIT JUICE AT TIME OF MANUFACTURE <sup>1</sup>				SERIES STORED	PER CENT ASCORBIC ACID RETAINED IN CANNED GRAPEFRUIT JUICE AFTER STORAGE—								
		SERIES				SERIES					SERIES								
		I				II					III								
		AVER.				AVER.					AVER.								
Mg per 100 ml										N.O.		3 MOS.		6 MOS.		9 MOS.		12 MOS.	
										per cent		per cent		per cent		per cent		per cent	
										Florida grapefruit juice, unsweetened									
12/29 <sup>2</sup>	4	40.9	—	—	—	40.9	96.8	—	—	96.8	96.0	87.9	80.9	73.5					
2/24	1	36.6	—	—	—	36.6	96.4	—	—	96.4	91.9	89.3	80.3	73.4					
3/5	2	41.0	45.3	42.0	—	42.8	98.0	90.9	90.0	93.0	93.2	82.7	78.1	73.7					
3/15	3	43.9	43.4	43.4	—	43.6	98.6	91.2	98.6	96.1	93.1	89.1	80.1	73.4					
3/17	7	39.8	39.6	39.6	—	39.7	99.0	100.0	97.5	98.8	93.4	89.7	81.5	75.6					
4/13	9	40.6	39.5	38.3	—	39.5	95.6	97.7	101.0	98.1	93.5	82.6	75.9	74.9					
4/22	11	43.1	46.8	40.5	—	43.5	95.6	87.8	101.0	94.8	93.2	85.1	77.8	74.9					
4/22	15	41.1	40.2	40.5	—	40.6	100.0	99.0	98.3	99.1	97.0	86.2	80.4	76.1					
4/26	10	39.9	36.9	39.2	—	38.7	91.7	97.6	96.4	95.2	90.9	87.0	82.2	77.1					
6/10	16	43.3	40.7	41.9	—	42.0	99.1	96.6	96.9	97.5	97.5	87.0	83.0	77.1					
										Florida grapefruit juice, sweetened									
12/30 <sup>3</sup>	5	40.4	—	—	—	40.4	97.3	—	—	97.3	97.5	88.5	80.4	72.6					
1/1	6	42.1	42.6	—	—	42.4	97.2	—	—	97.1	95.5	89.0	78.4	73.2					
4/7	8	41.1	—	—	—	41.1	97.1	—	—	97.1	95.5	89.0	78.4	73.2					
										Texas grapefruit juice, unsweetened									
1/6	24	39.8	37.8	38.9	—	38.8	94.7	97.9	95.1	95.9	95.2	84.9	77.2	71.6					
1/8	20	42.5	38.4	40.4	—	40.4	95.5	104.2	105.2	101.6	95.2	86.7	83.0	75.4					
1/19	28	38.5	39.6	40.5	—	39.5	114.3	98.2	98.3	103.6	93.2	84.7	77.4	70.9					
1/20	26	38.1	34.9	37.9	—	37.0	96.3	102.6	97.9	97.9	91.9	82.5	76.2	69.3					
2/15	21	45.5	45.7	45.4	—	45.5	97.1	97.8	97.4	97.4	92.5	80.5	74.7	68.3					
2/16	22	38.7	39.2	39.7	—	39.2	100.0	97.2	97.5	98.2	91.7	81.7	74.9	69.3					
2/17	25	40.6	43.5	43.3	—	42.5	98.0	98.2	95.2	97.1	92.7	82.0	74.8	68.2					
2/18	27	37.8	39.3	40.8	—	39.3	96.0	95.2	95.1	95.6	92.7	82.4	74.8	68.2					
3/23	23	39.3	37.7	38.1	—	38.1	95.4	98.1	98.1	98.1	92.7	81.6	73.5	68.2					
3/29	29	39.3	37.7	36.1	—	37.7	95.4	98.1	98.1	97.2	93.9	83.6	76.2	69.9					
										California-Arizona grapefruit juice, unsweetened									
1/28	40	44.0	47.0	48.0	—	46.0	102.3	105.9	103.6	100.8	94.3	92.2	84.1	74.3					
2/4	43	44.0	44.7	44.7	—	44.5	93.6	85.2	93.0	90.6	95.9	85.2	80.5	66.0					
4/15	43	42.0	44.7	43.0	—	43.0	99.8	98.2	99.5	99.6	93.0	89.0	91.1	85.3					
4/15	44	41.6	41.5	40.0	—	41.0	99.8	99.5	99.5	99.6	93.0	87.4	91.1	85.3					

SUMMARY TABLE 2

	ASCORBIC ACID FOUND IN SCREENED JUICE				PER CENT ASCORBIC ACID RETAINED IN CANNED GRAPEFRUIT JUICE AT TIME OF MANUFACTURE				PER CENT ASCORBIC ACID RETAINED IN CANNED GRAPEFRUIT JUICE AFTER STORAGE			
	NO. OF SERIES	MIN.	MAX.	AVER.	MIN.	MAX.	AVER.	3 MOS.	6 MOS.	9 MOS.	12 MOS.	
Florida	30	36.6	46.8	41.1	per cent	per cent	per cent	Min.	per cent	per cent	per cent	
Texas	19	38.7	43.0	40.9	87.9	101.0	98.6	Max.	90.5	94.0	85.0	
Cal. Ariz.	10	38.7	43.0	43.3	88.2	109.4	99.3	Aver.	85.4	78.7	73.1	
								No.	27	26	26	
All	72	34.9	48.0	41.1	85.2	114.3	97.7					

<sup>1</sup> Data on juice before screening, after pasteurizing and at other intermediate steps in manufacturing are not included because of small "over-all" loss, and to save space.

<sup>2</sup> Data from 3 series intermingled.

<sup>3</sup> A fourth series was run; screened juice, 45.3; per cent retained, 100.0.







**TABLE 5.—Per cent oil, suspended matter, and insoluble solids in factory and warehouse samples of grapefruit juice, orange juice, and blends.**  
Collected and examined 1942 and 1943

CODE MFR'S NO.	OIL	SUSPENDED MATTER BY CENTRIF.	INSOLUBLE SOLIDS	CODE MFR'S NO.	OIL	SUSPENDED MATTER BY CENTRIF.	INSOLUBLE SOLIDS
	per cent	per cent	per cent		per cent	per cent	per cent
<i>Florida grapefruit juice unsweetened</i>				1	.018	4	
4	.065	4	.041	1	.015	6	
1	.015	6	.053	<i>Blend of Texas grapefruit and orange juices, sweetened</i>			
2	.005	5		25	.035	9	
3	.012	4.5		<i>Florida orange juice unsweetened</i>			
7	.012	6		6	.033	14	
9	.018	3		1	.010	6	
11	.008	8		13	.045	10	
10	.005	5		17	.020		
16	.001			<i>Florida orange juice sweetened</i>			
<i>Florida grapefruit juice sweetened</i>				6	.027	10.5	
5	.002	5	.054	12	.035	11	
6	.005	7	.049	14	.008	10.5	
6	.010	8.5		18	.037	8	
8	.015	6.5		15	.015	6	
<i>Texas grapefruit juice unsweetened</i>				<i>Texas orange juice unsweetened</i>			
29	.010	4		25	.055		
24	.002	7		<i>California orange juice unsweetened</i>			
20	.010	4		45	.015	12	
28	.015	6		46	.018	8	
26	.005	6.5		40	.005	10	
21	.001	8		40	.005	10	
22	.010	6		46	.012	8.5	.148
25	.008	6	.064	46	.010	9.5	.133
27	.004	12.5		47	.005	10	.211
23	.015	7		47	.015	11	
<i>California and Arizona grapefruit juice unsweetened</i>				48	.015	11	
40	.001	6		48	.010	13	
40	.002	7		49	.008	12	.173
40	.005	7		49	.008	13	.156
41	.005	7		40	.003	10	.149
40	.010	8		40	.012	11	.156
42	.001	12	.159	47	.010	11	
43	.018	6	.026	48	.008	13	.199
43	.018	6		49	.003	12	
44	.008	8		49	.010	10	
<i>Blends of Florida grapefruit and orange juices sweetened</i>				50	.008	17	.268
6	.015	9		50	.005	18	
6	.035	9					
8	.020	8					

Methods of analysis:

Oil—*This Journal*, XXVII, 1, 201 (1944).

Suspended matter—As described in United States Standards for Grades of Canned Concentrated Orange Juice U.S.D.A. Bulletin (1943).

Insoluble solids—*Methods of Analysis*, A.O.A.C., p. 336, Fifth Ed. (1940).

recorded in Tables 2, 3, and 4. Such apparent gains are not unusual (5) in an investigation of this kind when the overall losses in the ascorbic acid content are small and the fruit being processed during the sampling operation varies with respect to source (such as stored packing house culls, freshly picked orchard run fruit, etc.), size, maturity, irrigation practice, and perhaps in other ways which may affect the ascorbic acid content. It was not possible to maintain exact identity of the juice at different stages in the manufacturing process, even though the times of sampling were planned to conform as nearly as possible to the rate of flow of the juice being processed. However, it is not believed that the variations due to this cause materially affected the overall averages of ascorbic acid retention recorded in the summaries in Tables 2, 3, and 4. Those averages check corresponding data reported in similar investigations (3) (4). We were not in a position to make an intensive study of each factor affecting the ascorbic acid content of the fruit being processed, nor was it considered that an extensive statistical study of the data was justified.

The percentages of ascorbic acid retained on storage, recorded in Tables 1, 2, 3, and 4, indicate that each of the citrus juices investigated retain about the same percentages of ascorbic acid on storage.

Sixty-eight factory and warehouse samples in sanitary cans (30 orange juice; 32 grapefruit juice; 6 blends) were collected in 1942 and 1943 and examined for ascorbic acid, volatile oil, soluble solids (by hydrometer, by refractometer, and by drying), insoluble solids by drying, suspended matter by centrifuging, acidity, pH, consistency, visible defects, flavor, dimensions of can, vacuum in inches, and fill of container. Soluble solids by drying and insoluble solids were not determined on all samples. For brevity, we are recording in Table 5 only the results on oil, suspended matter, and insoluble solids. The other data can be consulted in our files.

The data in Table 5 are largely self-explanatory. It is not claimed that they necessarily reflect trade practice. The average percentages of oil are: grapefruit juice 0.010%; orange juice 0.016%; blends 0.023%. The Cali-

TABLE 6.—*Texas grapefruit juice, 39 samples, early 1942*

	OIL	SUSPENDED MATTER	ASCORBIC ACID
	<i>Per cent</i>	<i>Per cent</i>	<i>Mg per 100 ml</i>
<i>Unsweetened, 36 samples (19 factories)</i>			
Minimum.....	0.002	3	32.0
Maximum.....	0.029	10	45.2
Average.....	0.014	6	37.8
<i>Sweetened, 3 samples (1 factory)</i>			
Minimum.....	0.007	7	31.6
Maximum.....	0.026	10	37.2
Average.....	0.015	8	35.0

California orange juices averaged 0.009% oil, and the Florida orange juices 0.026%.

Also, in January and February, 1942, 39 samples (36 unsweetened; 3 sweetened) of production line and warehouse samples of Texas grapefruit juice in sanitary cans were collected. These were analyzed in February and March of that year for soluble solids by refractometer and hydrometer, acidity, suspended matter, ascorbic acid, volatile oil, and pH. For brevity, only a part of these data is summarized in Table 6.

#### SUMMARY

(1) The average amounts of ascorbic acid in screened citrus juices and the average percentages retained during manufacture in 529 samples from 41 factories (925 determinations) were:

<i>Kind</i>	<i>Average ascorbic acid screened juice (Mg per 100 ml)</i>	<i>Per cent ascorbic acid retained</i>
Grapefruit juice	41.1	97.7
Orange juice	50.6	98.3
Blends	45.0	97.6

(2) The average percentages of ascorbic acid retained during storage at room temperatures, in 51 samples of citrus juices, were:

<i>Per cent ascorbic acid retained</i>			
<i>Months stored</i>	<i>Grapefruit juice</i>	<i>Orange juice</i>	<i>Blends</i>
3	94.5	92.5	93.3
6	85.4	85.3	86.1
9	78.7	80.1	77.9
12	73.1	75.0	72.2

#### REFERENCES

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- (4) WAGNER, J. R. *et al.*, *Food Research*, 10, 469 (1945).
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DETERMINATION OF PHENOTHIAZINE IN  
MEDICINAL PREPARATIONS

By VINCENT E. STEWART (Food and Drug Laboratory, State of Florida,  
Department of Agriculture, Tallahassee, Florida)

Methods for the determination of phenothiazine in medicinal preparations were reviewed, and published methods were investigated, by Stewart (1).

A collaborative study of the colorimetric method, by oxidation with bromine, was reported by Stewart (2), and two of the collaborators, McClosky and Smith (3) studied the use of colored filters in this determination. The method was adopted as a tentative procedure in the *Methods of Analysis* (4). Payfer and Marshall (5) reported a gravimetric method for the determination of phenothiazine in which the phenothiazine is precipitated as  $\text{Pt}(\text{C}_{12}\text{H}_9\text{NS})_2\text{Cl}_4$  by means of chloroplatinic acid.

The gravimetric method of Payfer and Marshall has been studied with the purpose of determining whether it is suitable as an adjunct to the present A.O.A.C. tentative method or whether it could be used to replace this method. The method was found to be satisfactory as described; however, it was found possible to so modify the method as to simplify the procedure and improve the accuracy, by the use of larger volumes.

The method described by Payfer and Marshall requires the weighed sample to be extracted in a Soxhlet extractor with alcohol (95 per cent). Alcohol is a relatively poor solvent for phenothiazine, whereas acetone is a good solvent. The National Formulary (6) states that one gram of phenothiazine dissolves in about 75 ml. of alcohol or in 5 ml. of acetone. The reaction of phenothiazine with chloroplatinic acid proceeds as well in acetone solution as it does in alcohol solution. When using acetone as the solvent for phenothiazine, solution can be effected merely by stirring the finely ground sample with the solvent, thus eliminating the Soxhlet extraction. It is possible to eliminate the Soxhlet extraction when using alcohol as the solvent, provided that the sample is thoroughly shaken with the alcohol, *e.g.*, about  $\frac{1}{2}$  hour in a mechanical shaker; and that the amount of phenothiazine is well below the maximum amount which can be dissolved by the alcohol. However, the ready solubility of phenothiazine in acetone obviates these difficulties.

Chloroplatinic acid solution can be prepared by the A.O.A.C. method which is used in preparing this reagent for the determination of potash by the Lindo-Gladding method. A solution containing the equivalent of 2.0 g platinum in 100 ml is satisfactory. Since an excess of hydrochloric acid in the reagent appears to have no effect on the determination, the hydrochloric acid need not be completely eliminated from the reagent.

Payfer and Marshall did not report any results obtained when the method was used with a pure grade of phenothiazine. In order to investi-

gate the reliability of the method the best grade of phenothiazine obtainable was repeatedly recrystallized from toluene, using activated charcoal, washed with petroleum ether, and dried in the oven at 100°C. The assay of this material was well within the limits of accuracy of the method.

#### METHOD

Powder the sample in a mortar if not already in the form of a powder. Weigh a portion of the sample equivalent to about 0.4 to 0.5 gram of phenothiazine in a 50 ml beaker. Stir the weighed portion thoroughly with acetone and pour the liquid through a retentive filter paper (e.g., Whatman #5), collecting the filtrate in a 100 ml volumetric flask. Continue to wash the residue in the beaker, and the filter, with small portions of acetone until the volume of the filtrate is nearly 100 ml, then dilute to volume with acetone. Transfer, by means of a pipet, a 25 ml aliquot of the acetone solution to a 100 ml beaker and add 7.0 ml of the chloroplatinic acid solution (which contains the equivalent of 2.0 g Pt in 100 ml). Stir the soln thoroly, add 15 ml of water with stirring and allow to stand several minutes. Filter the green precipitate on a fritted glass crucible (Porosity F), or a Gooch crucible with a tightly packed asbestos mat. Wash the precipitate into the crucible with acetone and scrub the beaker with a rubber policeman. Wash the precipitate in the crucible with several small portions of water and finally with acetone. Dry the crucible to constant weight in the oven at 100°C.

Wt. of precipitate  $\times 0.5416$  = wt. of phenothiazine

Each ml of the chloroplatinic acid solution is theoretically equivalent to about 41 mg of phenothiazine, but it is best to use a considerable excess of chloroplatinic acid solution in order to assure quantitative precipitation of phenothiazine. The filtrate should be yellowish-orange in color. The presence of a greenish coloration or a precipitate in the filtrate indicates that an insufficient amount of chloroplatinic acid, or of water, was added.

#### DISCUSSION

Several phenothiazine preparations, including both commercial preparations and mixtures of phenothiazine with various excipients prepared in the laboratory, were analyzed by the above method and by the colorimetric method described in *Methods of Analysis* (4). These samples were in most instances portions of samples which were analyzed by collaborators in the previous investigation of the colorimetric method (2). They included mixtures of phenothiazine with starch, lactose, and talc, two brands of commercial tablets, and two brands of commercial phenothiazine powder. The results obtained by the two methods agree very well. For example, a sample of medicinal grade phenothiazine, when analyzed by this method, gave the following assay: 99.8%, 99.9%, 99.9% (average 99.9%). The average result obtained by three different analysts, using the A.O.A.C. method, was 99.3%. A sample of commercial phenothiazine tablets, when analyzed by this method, gave the following assay: 73.5%, 73.6%, 73.1% (average, 73.4%). The average result obtained by three different analysts, using the A.O.A.C. method, was 73.6%.

The principle difficulties encountered with the gravimetric method

were: 1. The use of an insufficient excess of chloroplatinic acid. 2. The use of an insufficient amount of water in the reaction mixture. The precipitation is not quantitative unless the acetone is sufficiently diluted with water. The use of an insufficient amount of water results in the formation of additional precipitate in the filtrate. 3. The use of a filter lacking sufficient retentiveness. The main objection to the method is that it requires the use of chloroplatinic acid, an expensive reagent which must be recovered. This is not a serious objection since the platinum can be recovered from the precipitate by ignition. Excess platinum in the filtrate can be recovered by evaporation and ignition; however, because of the possibility of contamination from this source, the platinum should be purified by dissolving in aqua regia, precipitating ammonium chloroplatinate by the addition of ammonium chloride, and igniting the ammonium chloroplatinate.

The gravimetric method is a valuable adjunct to the colorimetric method described in *Methods of Analysis* (4). The choice of methods depends largely upon the individual preference of the analyst and the equipment which is available. In the event that interfering substances are encountered the analyst can check his results by the alternate method. Any highly colored substance may interfere to some extent with the colorimetric method, and substances which form insoluble complexes with chloroplatinic acid (e.g., alkaloids) would be expected to interfere with the gravimetric method.

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- (4) *Methods of Analysis*, A.O.A.C., 6th Ed., 1945, 39.175-7.
- (5) PAYFER, R., and MARSHALL, C. V., *This Journal*, **28**, 429-30 (1945).
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#### THE ANALYSIS OF A SUNBURN PREVENTIVE CREAM

By S. H. NEWBURGER\* (Cosmetic Division, U. S. Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

The Cosmetic Division of the U. S. Food and Drug Administration frequently has occasion to analyze various cosmetic creams. As sunburn preventive creams are becoming increasingly popular, it was decided to develop a system of analysis for one such cream to serve as a background for the investigation of other similar products.

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The formula of the cream chosen for this study is approximately that given in the "Draft of Proposed Quartermaster Corps Tentative Specification JCQD No. 1035, dated 29 June, 1945, for Cream, Sunburn Preventive," namely:

	% by weight
Glyceryl monostearate (self emulsifying).....	13.0
Lanolin.....	4.7
Propylene glycol.....	4.7
Titanium dioxide.....	2.5
Iron oxides—q.s. color	
Methyl para hydroxybenzoate.....	0.15
Isobutyl para aminobenzoate.....	2.0
Isopropyl myristate-palmitate.....	23.4
Water—q.s. 100%	

To evaluate the analytical methods for the cream, it was first necessary to determine the composition of certain of the materials used in its preparation. This paper is therefore divided into two parts; one dealing with the analysis of these materials; and the other, with the analysis of the cream itself.

#### I. MATERIALS USED IN THE PREPARATION OF THE SUNBURN PREVENTIVE CREAM

1. Lanolin—U. S. P. Anhydrous Lanolin
2. Titanium dioxide—A technical product
3. Iron oxides—A technical product
4. Propylene glycol—A technical product was fractionated at atmospheric and reduced pressures. The collected fraction assayed 100% by the periodate method (1).
5. Methyl para hydroxybenzoate—A technical product melting at 126–127°C.
6. Isobutyl para aminobenzoate—A technical product melting at 63.2–63.8°C (literature (2) 65°C) gave no elevation of the melting point on two recrystallizations. This material was assayed and used without purification.

#### ANALYSIS

Wells (3) bromometric assay for aminobenzoic acid esters was found applicable to the raw material. Proceed as follows: Weigh about 0.15 gm of isobutyl para aminobenzoate on a watch glass and wash into a 500 ml iodine flask with 20 ml of (1+1) HCl. After solution of the material in the acid, dilute to approximately 200 ml with water, and add from a buret, with vigorous shaking, 0.1 *N* potassium bromate-bromide solution until a slight excess of bromine is evidenced by a light yellow color. Stopper the flask and allow to stand for five minutes. Add an excess of potassium iodide solution through the well of the flask, avoiding loss of bromine, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate using starch solution as an indicator. 1 ml 0.1 *N* potassium bromate-bromide = 0.004831 gm of isobutyl para aminobenzoate. The average of three determinations on the product was 100 ± 0.1 %.

**Identification.**—The dibromo derivative prepared by Wells (3) is not very satisfactory because of its low melting point (74–75°C) and difficulty of recrystallization. The benzoyl derivative of isobutyl para aminobenzoate may be synthesized as follows:

Transfer 0.5 gm of isobutyl para aminobenzoate to a small glass stoppered flask and add 5 ml of water and 1 ml of benzoyl chloride. Then add 5 ml of 20% sodium hydroxide in small portions, shaking vigorously after each addition of alkali. Break up the coagulated precipitate with a glass rod and allow to stand for several minutes. Dilute the mixture with water, filter through a small Büchner funnel and wash precipitate thoroughly with water. Reprecipitate product from hot 75% alcohol and dry at 100°C. for 20 minutes. The compound crystallizes in fine white crystals. M.P. 127.8–128.2°C. (corr.). Theoretical nitrogen 4.71 per cent; found 4.65 per cent.

### 7. Isopropyl myristate-palmitate—The “Delyl Extra” grade.

#### ANALYSIS

Saponify samples of about 1.8 gm by the procedure described under the analysis of the cream. Determine the unsaponifiable matter, the fatty acids, and the equivalent weight of the fatty acids. After the extraction of the fatty acids filter the remaining aqueous alcoholic solution into a distillation flask, distill, and collect the first 5–10 ml of liquid. Test the distillate for isopropyl alcohol by the method given in U.S.P. XII under “Spiritus Frumenti.” The following results were obtained:

Unsaponifiable matter.....	0.16%
Fatty acids.....	84.5%
Equivalent weight of fatty acids.....	238
Test for isopropyl alcohol.....	positive

8. *Glyceryl monostearate (self emulsifying).*—The commercial self-emulsifying glyceryl monostearates are mixtures of a number of compounds. They contain free glycerine, mono-, di-, and tri-stearin as well as several per cent of the stearic acid soaps. The following analysis gives some of the constants of the material that was used.

#### ANALYSIS

Dissolve about 0.9 gm in 50 ml of chloroform and extract with three 20 ml portions of water acidified with a few drops of concentrated HCl. Reserve the chloroform solution. Combine the water extracts, wash with two 10 ml portions of chloroform and add washings to reserved chloroform solution. Filter the water extract, neutralize to methyl red, transfer to 100 ml volumetric flask, dilute to mark with water, and mix. Determine glycerine on a suitable aliquot by the periodate method (1). The result represents uncombined glycerine.

Filter the reserved chloroform solution through a cotton plug into a tared dish, evaporate the chloroform on the steam bath, dry in a 100°C. oven for 5–10 minutes, cool in a vacuum desiccator, and weigh as chloroform soluble material.

Dissolve the chloroform soluble material in benzene and saponify by the procedure described under the analysis of the cream. Determine the unsaponifiable matter, the fatty acids, the equivalent weight of the fatty acids, and the combined glycerine. The value for fatty acids represents both free and combined fatty acids. The following results were obtained:



	<i>per cent</i>
Uncombined glycerine.....	11.3
Chloroform soluble material.....	85.7
Unsaponifiable material.....	0.71
Fatty acids.....	76.0
(Equivalent weight of fatty acids.....	277)
Combined glycerine.....	12.7

## II. ANALYSIS OF THE SUNBURN PREVENTIVE CREAM

A cream of the following composition was prepared and analyzed:

	<i>% by weight</i>
Glyceryl monostearate (self emulsifying).....	14.04
Anhydrous lanolin.....	5.08
Propylene glycol.....	5.08
Titanium dioxide.....	2.70
Iron oxides.....	2.16
Methyl para hydroxybenzoate.....	0.16
Isobutyl para aminobenzoate.....	2.15
Isopropyl myristate-palmitate.....	25.22
Water.....	43.41
Total.....	100.00

### METHODS OF ANALYSIS

**Total ash.**—Weigh about 0.6 gm. of the cream into a deep 75 ml porcelain crucible. Evaporate the volatile material under an infra red drying lamp, char and burn off most of the carbon with a Meker burner, ash in a muffle oven at 750°C., cool, and weigh as total ash. Reserve ash.

**Titanium dioxide.**—To the crucible containing the total ash add 4 gm of  $\text{Na}_2\text{SO}_4$  and 8 ml of  $\text{H}_2\text{SO}_4$ , cover the crucible with a watch glass and heat on a hot plate until solution is complete. Cool and transfer to 100 ml volumetric flask containing 25 ml of cold water, cool, dilute to mark with water and mix. Determine the titanium colorimetrically (4) with  $\text{H}_2\text{O}_2$  in a suitable aliquot.

In this particular study the following procedure was used: To a 50 ml  $\frac{1}{4}$ " diameter test tube were added: a 2 ml aliquot of the soln, 7 ml of solvent (20 ml  $\text{H}_2\text{SO}_4$  and 10 gm  $\text{Na}_2\text{SO}_4$  diluted to 250 ml with water), and 1 ml of 3%  $\text{H}_2\text{O}_2$ . After mixing the soln, its transmission was measured by means of an Aminco Type "F" Photometer with No. 42 filter, and compared with the transmission of standard solns of titanium prepared by treating titanium dioxide in the same manner as the ash of the cream.

**Iron oxides.**—An approximation of the iron oxides can be obtained by subtracting the titanium dioxide from the total ash. The result for iron oxides will be in error by the amount of ash that is not iron oxides or titanium dioxide. This, however, should not be greater than 0.1 or 0.2 per cent.

**Propylene glycol and free glycerine.**—Weigh about 1.8 gm of the cream into a 50 ml beaker, add 10 ml of a (1+1) mixture of absolute alcohol and chloroform; heat with stirring until mixture boils, and decant through a Gooch crucible using suction. Re-extract the residue in the beaker with a 10 ml and then a 5 ml portion of hot (1+1) absolute alcohol-chloroform solution, and finally with 15 ml of hot chloroform, decanting each time through the Gooch crucible. Discard the residue. Transfer the extract to a separatory funnel with the aid of 25 ml of chloroform, and extract with a 40 ml aliquot of water acidified with one or two drops of HCl. Continue the extraction with a 10 ml and then a 5 ml aliquot of water. (Reserve the chloroform solution for the next determination.) Neutralize the combined aqueous extracts to

methyl red making the final adjustment with 0.02 *N* NaOH, filter through a cotton plug into a 100 ml volumetric flask, dilute to mark with water and mix. Transfer a 45 ml\* aliquot into a 100 ml volumetric flask, add 50 ml of 0.04 *N* KIO<sub>4</sub> (0.02 *M*), dilute to mark with water, mix, and allow to stand for one hour. Pipet 20 ml of the oxidized soln into a flask, dilute to 50 ml with water, add about 1 gm NaHCO<sub>3</sub> and 0.2 gm KI, and titrate the liberated iodine with standard 0.04 *N* arsenite soln using starch soln as an indicator. Standardize 10 ml of the 0.04 *N* KIO<sub>4</sub> with the standard 0.04 *N* arsenite soln by the same procedure. From the difference in the two titrations calculate the amount of periodate required to oxidize the 45 ml aliquot. Transfer another 45 ml aliquot into a glass stoppered flask, add the calculated amount of periodate plus 1–2 ml in excess, stopper the flask, allow to stand for one hour, add another drop of methyl red indicator and titrate to a clear yellow end point with 0.02 *N* NaOH.

1 ml 0.02 *N* NaOH = 1.84 mg glycerine

1 ml 0.04 *N* KIO<sub>4</sub> = 0.92 mg glycerine

1 ml 0.04 *N* KIO<sub>4</sub> = 1.52 mg propylene glycol.

Make the following calculations:

- a. Estimate the amount of glycerine from the alkali titration.
- b. Calculate the amount of periodate required to oxidize this amount of glycerine.
- c. Subtract the periodate required to oxidize the glycerine from the total amount of periodate that was required to oxidize the 45 ml aliquot and compute the remainder as propylene glycol.

The determination of glycerine by the alkali titration is a modification of the method advocated by Shupe (1). Shupe recommends running a blank on the KIO<sub>4</sub> solution for any acidity that may be present. It was observed that the KIO<sub>4</sub> solution used was acid to methyl red, and that the addition of 0.02 *N* NaOH<sub>4</sub> caused a slow unsatisfactory end point change that never became a clear yellow. On the other hand, KIO<sub>3</sub> solution is acid to methyl red, but gives a sharp change to a clear yellow on the addition of 0.02 *N* NaOH. That the unsatisfactory end point with KIO<sub>4</sub> was probably due to the KIO<sub>4</sub> itself was demonstrated in the following manner: To a large excess of propylene glycol in aqueous solution were added a few drops of methyl red indicator and enough 0.02 *N* NaOH to make the solution a clear yellow. When a solution of KIO<sub>4</sub> was added, the resulting solution turned red, However, as the periodate became reduced to iodate by the excess propylene glycol, the red color faded and the solution reverted to a yellow color. In the determination of glycerine by the alkali titration, the use of only a slight excess of periodate thus serves a twofold purpose: it eliminates the periodate blank and improves the end point.

*Isobutyl para aminobenzoate*.—Pass the reserved chloroform soln through four separatory funnels containing, respectively, 30, 20, and 10 ml of (1+1) HCl and 10 ml of water. (Reserve chloroform soln for the next determination.) Combine the (1+1) HCl and water extracts, dilute to about 200 ml with water, and filter through a cotton plug into a 500 ml iodine flask. Add 10 ml of 0.1 *N* potassium bromate-bromide soln, stopper the flask, shake well, and allow to stand for several minutes.

\* If 50 ml of periodate are insufficient to oxidize the 45 ml aliquot, a smaller aliquot should be used.

Drain excess potassium iodide soln into the flask and titrate the liberated iodine with 0.1 *N* sodium thiosulfate using starch soln as an indicator. One ml 0.1 *N* potassium bromate-bromide soln = 0.004831 gm of isobutyl para aminobenzoate.

(The extraction of isobutyl para aminobenzoate from chloroform was tested by dissolving the material in chloroform and following the above procedure. Three samples varying in weight from 45 to 142 mg gave an average recovery of 100  $\pm$  0.2%. When the sample size was increased to 250 mg., troublesome emulsions were encountered.)

*Anhydrous lanolin, glyceryl monostearate, methyl para hydroxybenzoate, and isopropyl myristate-palmitate.*—Filter the reserved chloroform soln through a cotton plug into a tared dish, evaporate the chloroform on the steam bath, dry in a 100°C. oven for 5–10 minutes, cool in a vacuum desiccator, and weigh as anhydrous lanolin, glyceryl monostearate, methyl para hydroxybenzoate, and isopropyl myristate-palmitate. Reserve residues for the lanolin determination.

*Anhydrous lanolin.*—Dissolve the above residue in 50 ml of benzene, transfer to a flask, add 25 ml of absolute alcohol and 1 gm of KOH, and reflux for 2 hours. Transfer the saponified material to a separatory funnel, add 50 ml of hot water, shake well, and draw off the aqueous layer. Extract the aqueous soln with two more 20 ml portions of hot benzene. (Reserve the aqueous soln for the fatty acid determination.) Combine the benzene extracts, wash with several 10 ml portions of 30% alcohol, and add washings to reserved aqueous soln. Filter the washed benzene extract through a cotton plug into a tared dish, evaporate benzene on steam bath, dry in a 100°C. oven for 5–10 minutes, cool in a vacuum desiccator, and weigh. This weight multiplied by the factor 1.96 approximates the anhydrous lanolin. (On a number of samples of anhydrous lanolin, it was observed that the unsaponifiable portion was about 51% of the sample.) The result for lanolin will be high if any of the other materials contain unsaponifiable matter.

The weighed material can be checked qualitatively for lanolin by the Liebermann-Burchard color test. Dissolve a portion of the material in 10 ml of chloroform and add 4 ml of acetic anhydride and several drops of H<sub>2</sub>SO<sub>4</sub>. A green color is a positive test for lanolin.

*Fatty acids.*—Acidify the reserved aqueous soln with HCl and extract with three 20 ml portions of chloroform. (Reserve the acid aqueous soln for the combined glycerine determination.) Wash the combined chloroform extracts with several 10 ml portions of water and add washings to reserved aqueous soln. Filter the washed chloroform extract through a cotton plug into a tared beaker, evaporate chloroform on a steam bath, dry in 100°C. oven for 5–10 minutes, cool in vacuum desiccator and weigh as fatty acids. Reserve fatty acids.

*Equivalent weight of fatty acids.*—Dissolve the fatty acids in ethyl alcohol (neutralized to phenolphthalein with alkali), and titrate to the phenolphthalein end point with 0.1 *N* NaOH. Calculate the equivalent weight of the fatty acids.

*Combined glycerine.*—Filter the reserved aqueous soln through a cotton plug, evaporate to about 25 ml on the steam bath, carefully neutralize to methyl red indicator with alkali and analyze for glycerine by the periodate method (1).

*Water.*—Water can be most accurately estimated indirectly. Add the percentages of "Total ash," "Propylene glycol and free glycerine," "Isobutyl para aminobenzoate," and "Anhydrous lanolin, glyceryl monostearate, methyl para hydroxybenzoate, and isopropyl myristate-palmitate." Subtract this total from 100% and the remainder is the percentage of water.

(Water can be determined directly by xylene distillation. However, the propylene glycol will also distill with the water. The substitution of a lower boiling liquid such as heptane does not help. Accordingly the volume corresponding to the amount of the previously determined propylene glycol must be subtracted from the total volume. Experiment showed that the volumes of propylene glycol and water

are additive when mixed in the ratio of 1:11 by weight. The percentage recovery of water was about 96%.)

**Qualitative test for methyl para hydroxybenzoate.**—To about 2 gm of cream in a 100 ml beaker, add 3 ml of absolute alcohol and 2 drops of concentrated HCl, and heat to boiling on a hot plate. Remove beaker from the hot plate, cool, add 50 ml of ether with stirring, filter through Gooch crucible with suction, and transfer filtrate to separatory funnel with 25 ml of ether. Discard residue. Extract ether soln with four 25 ml portions of (1+1) HCl. Discard (1+1) HCl extract and wash ether soln with two 5 ml portions of water. Transfer ether soln to beaker, evaporate ether on steam bath, treat residue with 20 ml boiling methanol, stir vigorously, cool, and filter. Add 10 ml of water, and one drop of HCl to the methanol soln and extract with three 20 ml portions of petroleum ether. Discard the petroleum ether extracts. Evaporate methanol soln to a few ml on the steam bath, dilute with 20 ml boiling water, cool, transfer to separatory funnel, and extract with three 15 ml portions of ether. (This additional extraction removes any remaining HCl which would inhibit the development of color in subsequent steps.) Combine the ether extracts and wash with 5 ml portions of water until washings are neutral to litmus paper. Evaporate ether on steam bath, take up residue in 20 ml boiling water, transfer to 50 ml test tube, cool, add 2 ml of Millon's reagent\* and immerse in a boiling water bath for 5 minutes. A rose color indicates the presence of methyl para hydroxybenzoate. (Isobutyl para aminobenzoate also gives a positive test with Millon's reagent and must be completely removed by the HCl extraction.) Efforts to modify the above procedure to obtain quantitative recoveries were unsuccessful. A recovery of 50% was the best that could be obtained.

The method separating fatty materials from methyl para hydroxybenzoate by the use of petroleum ether and about 60% methanol should be generally applicable to a number of cosmetic preparations.

#### RESULTS OF ANALYSIS

	Found per cent	Theoretical per cent
Ash 750°C.	4.98	
Titanium dioxide	2.71	2.70
Iron oxides (by difference)	2.27	2.16
Free glycerine	1.54	1.59
Propylene glycol	5.17	5.08
Isobutyl para aminobenzoate	2.17	2.15
Anhydrous lanolin		
Glyceryl monostearate	42.0	42.91
Isopropyl myristate-palmitate		
Methyl para hydroxybenzoate		
Unsaponifiable matter	2.71	
Anhydrous lanolin	5.31	5.08
Fatty acids	34.2	34.7
(Equivalent weight of fatty acids	252.3)	
Combined glycerine	1.74	1.78
Water (by difference)	44.14	43.41
Methyl para hydroxybenzoate	present	0.16

\* Millon's reagent: One part by weight of mercury is dissolved in twice its weight of concentrated nitric acid with gentle warming. The resulting solution is diluted with twice its volume of water.

In computing the theoretical value for the total percentage of anhydrous lanolin, glyceryl monostearate, isopropyl myristate-palmitate, and methyl para hydroxybenzoate, the percentage of uncombined glycerine in the glyceryl monostearate (1.59%) was not included.

The theoretical value for the fatty acids was estimated from the analysis of the glyceryl monostearate and the isopropyl myristate-palmitate ingredients, and from the fact that previous work had indicated that lanolin contains approximately 51 per cent of fatty acids. The contribution of the hydroxybenzoic acid from its methyl ester was also included in the estimate.

*Conclusion.*—A detailed analysis has been developed for a "Sunburn Preventive Cream." It is believed that this analysis will serve as a general outline for development of procedures for analysis of similar products.

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## NOTE

### A Note on the Adulteration of Shredded Coconut with Papaya Stem Tissue

By WILLIAM V. EISENBERG (Washington, D. C.) and ROBERT E. O'NEILL  
(Atlanta, Georgia), U. S. Food and Drug Administration

Our attention was recently called to the adulteration of desiccated shredded coconut with papaya stalk tissues (*Carica papaya* L.). The sample of imported shredded coconut that was subjected to routine microscopic examination showed the presence of tissues that were foreign to the cellular content of shredded coconut. The tissues in question consisted of thin-walled rounded parenchymal cells containing occasional rosette crystalline aggregates of calcium oxalate and xylem reticulate ves-

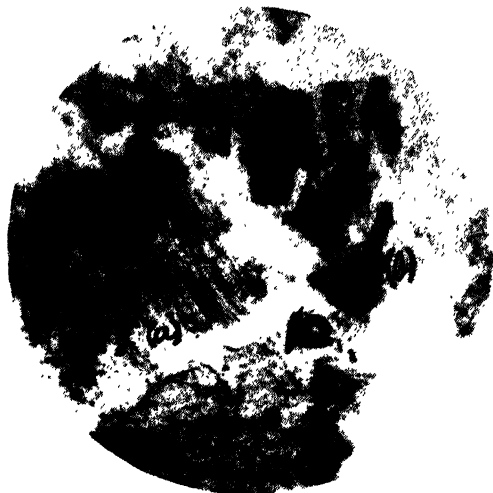


FIG. 1.—Shredded coconut adulterated with papaya stem tissues: (a) coconut endosperm cells; (b) reticulate vessel from papaya stem xylem.  $\times 58$ .

sels (Figs. 1 and 2). The calcium oxalate rosettes were usually 45 to 65 microns in diameter and the vessels were up to 300 microns for the larger forms.

None of these elements are found in shredded coconut, which consists almost wholly of the isodiametric and radially elongated cells of the endosperm, with only contents. Fragments of the spermoderm or outer brown skin of the coconut meat, consisting of large elongated cells with porous walls in the outer layer and small nonporous cells in the inner layers, are infrequently observed. The presence of the vascular vessel elements, and cells with calcium oxalate rosettes, could therefore be attributed only to the addition of some adulterant.

Microscopic comparison of the questionable tissue with the fleshy xylem and pith tissues from the stem of *Carica papaya* showed the two to be identical (Fig. 3). The particles of questionable material were fleshy and white, and to the unaided eye seemed quite similar to the fragments of shredded coconut. However, under the Greenough type microscope at a magnification of about  $30\times$ , the questionable particles exhibited a yellowish cast due to the vascular elements which contrasted with the



FIG. 2.—Papaya stem tissue isolated from sample of adulterated shredded coconut: Reticulate vessels, xylem parenchyma, and crystal cells each containing a rosette of calcium oxalate are shown.  $\times 75$ .

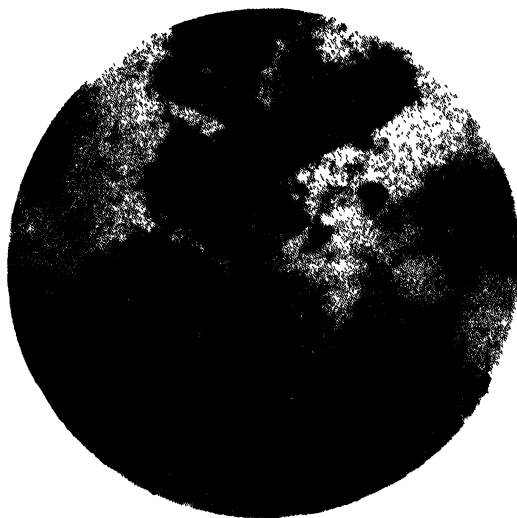


FIG. 3.—Papaya stem xylem tissues shredded from authentic material: Reticulate vessels and xylem parenchyma are shown.  $\times 58$ .

snow white appearance of the coconut. By immersing the material in 60 per cent alcohol in a flat dish and searching under the Greenough microscope, the questionable pieces can easily be removed for further examination with the compound microscope.

The presence of crystal cells containing rosettes of calcium oxalate, and of reticulate vessel elements, serves to distinguish readily coconut endosperm tissue from papaya stem tissue. Crystal cells are characteristic of the papaya plant generally, being also found in the fruit tissues. In contrast, the coconut is devoid of calcium oxalate rosettes in any part of the fruit, or of the developing embryo. Vessel elements are found in parts of the coconut fruit other than the fleshy endosperm. However, those from the developing cotyledon in sprouted coconuts are of the spiral type, while the straw-like fibrous mesocarp of the coconut fruit which contains reticulated vessels is of such texture and appearance that it cannot be confused with the fleshy white endosperm.

## BOOK REVIEWS

**The Control of Insects in Flour Mills.** By J. A. FREEMAN and E. E. TURTLE. Published by H. M. Stationery Office, London, 1947, 76 pages, index, full page plates and text figures. Price \$2.35.

The bulletin covers material similar to that in many publications in this country but has several unique features that make it a useful addition to its field, and perhaps of special interest in cases where control measures must be undertaken on a small scale by relatively inexperienced operators. Attention is given to details of sanitation, often overlooked by entomologists but which are finding increased attention from regulatory officials, such as bag cleaning, mill hygiene, modern self cleaning design, cleaning and sweeping, elimination of unnecessary accumulations of grain residues and trash. The section on "life histories and habits" includes an introduction of general entomological information, and material on the parasitic wasps.

Aside from being written on a public appeal level, and the sanitation and grain pest parasite sections, there is little to recommend this pamphlet at its price over the standard works already available more cheaply in this country.

KENTON L. HARRIS

**Two Blades of Grass—A History of Scientific Developments in the U. S. Department of Agriculture.** By T. Swann Harding. University of Oklahoma Press, Norman, Okla. (1947) 6×8½", 352 pp. 24 illus. Price \$3.50.

This is a history in the best and most modern sense of the term. When the author, in his introduction, calls it a popular presentation, he does not mean that it is highly dramatized or over-simplified, after the manner of those books which so nauseate the scientifically trained reader; it is nothing of the sort.

The author has had a practically life-long connection with the Department of Agriculture, first as a scientist in his own right in the old Bureau of Chemistry and later in the Bureau of Animal Industry, second as editor of scientific publications, and finally as editor of the departmental house organ, "U.S.D.A.," with which many of our readers are familiar. In the latter capacity he has actually visited virtually all of those departmental agencies, both in Washington and in the field, about which he writes; he has discussed, at length and with real understanding and sympathy, the various projects with those who were actually doing the work; he has delved deeply into the historical background of the Department from the feeble beginnings in the Patent Office in 1836; he has read the important scientific papers and



bureau reports of the earlier years; he is thoroughly familiar with the present complex organization of the Department, with its gradual evolution, and with the way its work has been, and is, interwoven with that of other governmental agencies, State and Federal. It is no wonder, therefore, that he has produced a book which everyone with either a professional or a cultural interest in Agriculture will wish to own. With his easy, informal style and his gift of selection, condensation, and synthesis, he has managed to pack more useful, yes, and inspiring, information into one volume, than in this reviewer's opinion, can be found between the covers of any other single book on the subject. It is a pleasure to call attention to the index, which was prepared by an Agricultural indexer who knew her business. One can locate in it, at a glance, all of the important names and projects down through the years. Such an index, to this reviewer, adds enormously to the value of a book which is literally crammed with useful information, and with references to important source material not readily accessible even to the specialist seeking information outside of his own familiar field.

After five brief preliminary chapters which deal with early origins and high spots, and which give vignettes of some of the earlier scientists with and for whom the author worked, the succeeding chapters deal with cooperative relations with the State Experiment Stations, with animal industry, human nutrition and home-making, soils and their conservation, the dairy industry, agricultural engineering, weather, roads, wild life, food and drug administration, and the scientific publications of the Department.

In each chapter the author weaves together a number of threads so successfully that, in the compass of from a dozen to 3 dozen pages, he has managed to produce a firm tapestry. We have a brief account of the important researches, a vivid vignette of the worker himself; a resume of the social and monetary implications of the work, and of its interconnection with other studies in or out of agriculture; and numerous interesting sidelights which make for unity and perspective.

Let the scientific reader not be misled by these novel-like chapter titles: these are merely "come-ons" to the non-scientific reader!

The faults of the book are decidedly minor and might not even strike another reviewer as faults. The illustrations are excellent and well-chosen, but they could have been placed in closer relationship to the text; and there are many places where the lowly comma might have been used to advantage in the interest of smooth and rapid reading.

The book has a brief but warmly commendatory foreword by Secretary Clinton P. Anderson. Its dedication by the author is worthy of quotation:

"Dedicated to those sincere, kindly, self-effacing, unavaricious humanitarians of highest integrity, the scientists who made the Department of Agriculture great as a research institution, and to my colleagues in information work in the various agencies of the Department, who so cheerfully and generously helped me to gather together the material comprising this book."

W. B. WHITE

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